ABSTRACT OF THESIS

Name of Candidate Diane Bannister

Address MRC-MGRU, Department of Molecular Biology, King's Buildings, EDINBURGH 9. EH9 3JR

Degree Doctor of Philosophy *Date* August 1969

Title of Thesis Restriction and modification controlled by resistance transfer factors.

A total of 153 R^+ strains of Escherichia coli were surveyed with eight bacteriophages. Of the 153 R^+ strains, 61 reduced the efficiency of plating (e.o.p.) of one or more bacteriophages; the 61 strains could be placed in ten groups on the basis of the pattern and level of reduction in e.o.p. observed, but only six of these groups were associated with transmissible determinants. Two of the remaining groups, termed I and II, restricted and modified five of the test phages, λ, ø80, P1, P2 and T1, i.e. determined a host specificity. In two of the remaining four groups, a reduction in e.o.p. of the phage BF23 was associated with resistance to colicin Ib. The possibility is discussed that these two, and the two other groups represent cases of restriction without modification.

The possible connection between fi character (repression of F-factor expression), resistance to tetracycline (Tc), and host specificity determinants was explored for the R factors of groups I and II. All group II strains carry fi^- R factors associated with the resistance determinants, whereas the group I R factor is fi^+. There is no specific relationship between Tc resistance and host specificity carried by R factors. One group II strain was further investigated and the suggestion made that this strain harboured two fi^- sex factors, at least one of which is an R factor.

Restrictionless mutants of two phenotypes, r^-m^- and r^-m^+, were isolated from R factors of both group I and group II. The host specificities of the two groups were different from the host specificities of E.coli K, B, and 15, and from that of bacteriophage P1. No complementation was detected between restrictionless mutants of the R factors and intact host specificity determinants of E.coli K and B, and bacteriophage P1. It is suggested that the R factor associated host specificities are, however, similar in genetic structure and operation to those of E.coli K and B.

Use other side if necessary.

RESTRICTION AND MODIFICATION CONTROLLED BY RESISTANCE TRANSFER FACTORS

by

Diane Bannister, B.A. (Oxon)

A thesis presented for the Degree of Doctor of Philosophy

Department of Molecular Biology

University of Edinburgh August 1969

BIBL. UNIV. EDINB. GEN. EX

A B S T R A C T

A total of 153 R^+ strains of Escherichia coli were surveyed with eight bacteriophages. Of the 153 R^+ strains, 61 reduced the efficiency of plating (e.o.p.) of one or more bacteriophages; the 61 strains could be placed in ten groups on the basis of the pattern and level of reduction in e.o.p. observed, but only six of these groups were associated with transmissible determinants. Two of the remaining groups, termed I and II, restricted and modified five of the test phages λ, ø80, P1, P2 and T1, i.e. determined a host specificity. In two of the remaining 4 groups, a reduction in e.o.p. of the phage BF23 was associated with resistance to colicin Ib. The possibility is discussed that these two, and the two other groups represent cases of restriction without modification.

The possible connection between fi character (repression of F-factor expression), resistance to tetracycline (Tc), and host specificity determinants was explored for the R factors of groups I and II. All group II strains carry fi^- R factors associated with the resistance determinants, whereas the group I R factor is $fi^\pm$. There is no specific relationship between Tc resistances and host specificity carried by R factors. One group II strain was further investigated and the suggestion made that this strain harboured two fi^- sex factors, at least one of which is an R factor.

Restrictionless mutants of two phenotypes, r^-m^- and r^-m^+, were isolated from R factors of both group I and group II. The host specificities of the two groups were different from the host specificities of E. coli K, B and 15, and from that of bacteriophage P1. No complementation was detected between restrictionless mutants of the R factors and intact host specificity determinants of

E. coli K and B, and bacteriophage P1. It is suggested that the R factor associated host specificities are, however, similar in genetic structure and operation to those of *E. coli* K and B.

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

CHAPTER 2

MATERIALS AND METHODS

CHAPTER 4

CHAPTER 5

ISOLATION AND PROPERTIES OF RESTRICTIONLESS MUTANTS OF RI AND RII

CHAPTER 1

INTRODUCTION

SECTION I

HOST CONTROLLED MODIFICATION

A. Early Observations

The conflict between the evolutionary theories ascribed to Lamarck and Darwin survived in the field of bacteriology until recent decades. The ease with which bacterial cultures adapt to initially unfavourable growth conditions was taken as strong evidence for the Lamarckian viewpoint which claimed that this adaptability was due to the direct influence of the environment on the bacteria. Introduction of the fluctuation test (Luria & Delbrück, 1943), and the concept of clonal analysis (Newcombe, 1949), preceded the rapid accumulation of evidence. The "adaptability" of bacterial cultures was accepted as due to the selection of mutants pre-existing in the bacterial population.

Ironically, in the early 1950's there were four independent reports of non-heritable variations in the host range of a bacteriophage (Anderson & Felix, 1952; Luria & Human, 1952; Bertani & Weigle, 1953; Ralston & Kreuger, 1954). In agreement with the Lamarckian doctrine, the host range of a bacteriophage depended on the last host (the environment) and required only a single cycle of growth to alter the host range. This phenomenon has been termed host controlled modification, host controlled variation and host-induced variation. From now on it will be referred to as host controlled modification.

The host controlled modification of phage λ, first observed by Bertani & Weigle (1953), is illustrated in Table 1.1. The three strains of Escherichia coli used, K12, B and C will be simply referred

Table 1.1.

The host controlled modification of bacteriophage λ

Last host	Efficiencies of plating on strains		
	C	B	K
C	1.0	$1x10^{-4}$	$4x10^{-4}$
B	1.0	1.0	$5x10^{-4}$
K	1.0	$2x10^{-4}$	1.0

to as K, B and C. Phage λ grown on C(λ.C) has an efficiency of plating (e.o.p.) on C which is defined as 1.0. When λ.C is plated on K, the e.o.p. is very much less than 1.0, and K is said to restrict λ.C, i.e. K is a restricting host. Phage λ.K, however, has an e.o.p. of 1.0 on both K and C, so that the λ is host modified. Just as λ.C is restricted and modified by K, it is also restricted and modified by B, illustrating the fact that a phage may carry the modification of any one of a number of strains. These modifications are specific, phage λ.K is still restricted on B, phage λ.B is likewise restricted on K. Strains which do not restrict phage are termed permissive.

A different host controlled modification was observed by Luria & Human (1952). They found that if the phage T2 was grown for a single cycle in the strain $B/4_o$ (a mutant of B resistant to the phages T3, T4 and T7), the progeny phage, designated T*2, were restricted on B and on $B/4_o$ itself, but not on a Shigella strain. The rare T*2 which successfully infected B gave rise to progeny indistinguishable from the original T2.

B. DNA as the Carrier of the Modification

i Injection of bacteriophage DNA

A change in the host range of a bacteriophage after a single cycle of growth in a restricting host could be due to selection of mutant phages. It is the reversion of this change by a single cycle of growth in a permissive strain which defines the host modification of phages as non-heritable. For example, λ.C is restricted by K, and the rare progeny λ.K which appear could be mutants selected by plating on the K host. When, however, the λ.K is restored to λ.C by growth in C, there has been no selection pressure, since C is permissive, so the λ.C can not be mutant phage. Since a change in host specificity is not inherited, it cannot involve a change in the genetic information carried by the phage DNA, and must therefore be due to an alteration in a phage component. In an attempt to identify the component affected, a number of phage properties were measured: inactivation by temperature, UV or specific anti-sera; adsorption; latent period; burst size, and appearance under the electron microscope were all unaltered by modification (Bertani & Weigle, 1953; Shedlovsky & Brenner, 1963).

Proof of the normal injection of restricted phage DNA required use of a technique developed by Hershey & Chase (1952). Specific labelling of phage DNA is achieved by use of the isotope ^{32}P, radioactive phage is adsorbed to the host under test, and then the phage coats are blended away from the cells. Continued association of the radioactivity with the cells indicates successful injection of the phage DNA. Lederberg (1957) was thus able to demonstrate the injection of the non-modified phage T1 DNA into P1 lysogens, which restrict phage T1. Similarly, T*4 was shown to be injected into

restricting hosts (Fukasawa & Saito, 1963). Good injection of phage λ DNA is also implied in experiments where λ.K is restricted by K(P1) (Dussoix & Arber, 1962). The same conclusion, that the restricted phage DNA is successfully injected, can also be reached from experiments designed to detect the presence of genes from a phage in a restricting host. Phage λ genes are detected by complementation (Terzi, 1968), and by marker rescue (Dussoix & Arber, 1962), while genes from restricted phage T*2, T*4 or T*6 have been detected by the direct measurement of phage-specific enzyme production (Fukasawa & Saito, 1963; Fukasawa, 1964a; Hattman, 1964; Hattman, Revel & Luria, 1966).

ii Association of parental DNA with parental host specificity in progeny phage

Only DNA enters the cell from a bacteriophage, with the exception of about 3% of the total protein. Since injection of restricted DNA is normal, two conclusions can be drawn; firstly that restriction is a process occuring after DNA injection, and, secondly, that it is the phage DNA which must carry the modification. Although a number of experiments imply that conserved parental DNA is associated with the parental host specificity (Ihler & Meselson, 1963; Christensen, 1964; Keller, 1964), the clear association of parental DNA molecules and host specificity was only demonstrated in experiments where the progeny phage particles can be separated into those carrying new DNA and those retaining parental DNA. This can be achieved by using modified phage labelled with a heavy isotope, for example, deuterium, to infect a none modifying host in light medium. Progeny phage particles containing new (light), semi-conserved (hybrid) and conserved (heavy) DNA molecules have different bouyant densities and

can be separated on a density gradient. Arber and his colleagues using phage λ.K(P1) in K found that not only is parental host specificity associated with conserved parental DNA molecules, but also with semi-conserved DNA molecules (Arber & Dussoix, 1962; Arber, Hattman & Dussoix, 1963). Clearly modification is associated with the DNA molecules, and modification of one strand of the duplex is sufficient to protect against restriction. These results were confirmed for phage fd (Arber, 1966), phage λ.K, and phage λ which carries both K and B modifications (Kellenberger, Symonds & Arber, 1966), and for T2 (Boyle, Ritchie & Symonds, 1965).

iii Host specificity of isolated DNA

Dussoix & Arber (1965) utilised a method of infecting Escherichia coli with purified λ DNA molecules, after making the cells competent by prior infection with phage λ (Kaiser & Hogness, 1960; Kaiser, 1962), to test whether host specificity is retained by phenol-extracted DNA. The DNA from phage λ.K(P1) not only retained the host specificity of the phage, but also retained it after changes in ionic strength, and pH, and after more specific treatments with protease and ribonuclease. The replicative form (RF) of the F specific phage fd can infect spheroplasts of Escherichia coli (Benzinger, 1968), and since the spheroplasts of B, and of P1 lysogens, retain the ability to restrict the fd RF, Linn & Arber (1968) utilised this method to show the continued association of the host specificity imposed by B and P1 with the extracted fd RF. It is clearly the phage DNA which carries modification.

C. The Nature of Modification

i Glucosylation of the bacteriophages T2, T4 and T6

The DNA of the T-even phages (T2, T4 and T6) differs from that of Escherichia coli in two respects. Firstly, the base 5-hydroxymethyl cytosine (HMC) replaces the base cytosine in the DNA. Secondly, the phage DNA contains glucose residues, which are attached only to the HMC, and are apparently added to the DNA after replication, since the intact DNA is the substrate for in vitro glucosylation (Kornberg, Zimmerman, Kornberg & Josse, 1959; Zimmerman, Kornberg & Kornberg, 1962).

Symonds, Stacey, Glover, Schell & Silver (1963) were the first to suggest that absence of glucosylation prevented the growth of the phages T*2 and T*6 on B. The original B/4 mutant was seen to be a typical uridine diphosphoglucose (UDPG) deficient mutant, displaying a number of metabolic abnormalities, which include the inability to ferment galactose (Sundarajan, Rapin & Kalckar, 1962; Hattman & Fukasawa, 1963), and failure to incorporate galactose into the cell wall polysaccharide, causing UDPG-deficient strain, of B but not of K, to be resistant to the phages T3, T4 and T6 (Shedlovsky & Brenner, 1963). Since UDPG is the glucose donor in the glucosylation of T-even DNA (Kornberg et al., 1959), T-even phages grown on UDPG deficient strains should have no glucose on the DNA; this is confirmed by chemical analysis (Fukasawa & Saito, 1963; Hattman & Fukasawa, 1963; Shedlovsky & Brenner, 1963) and by the change in bouyant density between the glucosylated and non-glucosylated phages (Erikson & Szybalski, 1964).

The formation of the glucosidic bond between the glucose and the HMC requires the participation of a phage-specific glucosyl

transferase. Mutants of the phages T2 and T6 have been found which are defective in production of the enzyme α-glucosyl transferase, and as expected, these T2gt and T6gt mutants, as they are termed, are incapable of glucosylating the phage DNA (Revel, Hattman & Luria, 1965). That the non-glucosylated T2gt or T6gt phages are restricted by B is ample confirmation that glucosylation is the mechanism involved in the modification of the T-even phages.

ii Methylation

Since the modification of phage λ DNA survives extraction from the phage, and a variety of treatments after extraction (see chap. 1, section I.B.iii), the modification is the addition of a covalently bonded molecule to the DNA. It is also apparent that modification can be applied to the DNA molecule itself, since it was acquired by DNA which had not been replicated (Arber & Dussoix, 1962; Kellenberger et al., 1966). Further, when a thymine requiring strain, already infected with phage λ, was superinfected with phage P1 and starved of thymine, the phage λ already formed received the P1 modification (Stacey, Symonds, Glover & Schell, 1963).

The methyl group has been detected in minor amounts in a number of bacterial strains (Dunn & Smith, 1958). Gold & Hurwitz isolated methylating enzymes and demonstrated that these enzymes will use as substrate only double stranded native DNA from another strain (Gold & Hurwitz, 1963a,b). Only if the DNA of a relaxed strain is made under conditions of limiting methylation can it be methylated by enzymes from the same strain (Gold & Hurwitz, 1963a), and this species specificity of the methylating enzymes led to analysis of bacterial and phage DNA in the hope of relating host specificity to a changed methylation pattern. While the Escherichia coli strains B and K have

a marked difference in the ratio of the bases found to be methylated, 6-methylaminopurine (MAP) and 5-methylcytosine (MC), this is not related to host specificity. The DNA of B and any phage grown on it contains no significant amounts of MC, unlike DNA from K (Klein, 1965; Klein & Sauerbier, 1965; Gough & Lederberg, 1966), but if the two host specificity regions are in otherwise identical strains, then there is no difference in the total methylation or the ratio of MAP to MC (Ledinko, 1964; Gough & Lederberg, 1966; Lederberg, 1966; Arber, 1968).

Considering the number of methyl groups on the phage λ.K, or λ.C or λ.B chromosome, for example, which is in excess of 100; and the error in estimation of methyl groups in DNA, which is about 10-20%, it is not surprising that a change of even 10 in the total number of methyl groups would not be detected. Though the total number of sites on the λ chromosome which can be modified by either the K or B modification enzymes is not known, it is probably much less than 10 (see chap. 1, sections I.D.iii and I.F). Direct evidence that methylation is involved in modification is lacking for phage λ, but there is some circumstantial evidence for it.

Although Arber (1965a) showed a reduced modification of phage λ in K(P1) in the absence of methionine only, the involvement of methionine in initiation of polypeptide chain synthesis invalidates any conclusion concerning methylation and modification from this experiment. The failure of Stacey et al. to observe any effect of methionine analogues on modification is also inconclusive. Evidence of a more convincing nature is founded on the initial observation that S-adenosylmethionine (SAM) is the methyl donor in methylation of both DNA and RNA (Gold & Hurwitz, 1963b). Klein & Sauerbier (1965) utilised the production by T3 of an enzyme cleaving S-adenosylmethionine

(Gold, Hausmann, Maitra & Hurwitz, 1964; Hausmann & Gold, 1966; Gefter, Hausmann, Gold & Hurwitz, 1966) to block the donation of methyl groups from SAM. A reduction was found in infected bacteria yielding modified phage T1 in a mixed infection of phages T1 and T3 in P1 lysogens. Hirsch-Kauffman & Sauerbier (1968) made a similar observation on mixed infections of K, B and C (P1) with phages T3 and λ.

In contrast to the circumstantial evidence available for phages λ and T1, it has been possible to demonstrate a change in methylation as a result of modification for the F specific phage fd. The phage fd has the advantage of a very low level of methylation when grown in non-modifying strains, the phage genome having only about 1.5 to 2 residues of MAP. In contrast, the modified phage fd.B has between 3 and 4 MAP per phage genome, so the increase in total methylation is about 100%, and therefore measureable. Supplementary evidence is supplied by the fact that phage fd mutants which are not restricted by B still only have about 1.4 MAP residues per phage genome even when grown in B. At least for phage fd, modification involves methylation, apparently of an adenine residue in the 6- position (Arber, 1969).

D. Restriction

i Effects of physiological conditions

It was early recognised that environmental conditions could dramatically alter the extent to which a bacterial culture restricted a phage (Bertani & Weigle, 1953; Luria, 1953). In general, restriction is not permanently impaired; recovery may be achieved by normal growth or a second change in the environment (Schell & Glover, 1965, 1966a; Uetake, Toyama & Hagiwara, 1964). Hence it may be

concluded that loss of restriction as seen in a bacterial population is not a selection of mutant bacteria. On the contrary, the evidence supports the hypothesis that the e.o.p. of a phage will be dependent on the fraction of phenotypically permissive cells in the bacterial culture, though other factors must also influence e.o.p., since related phages may have very different e.o.p. on the same culture (Eskridge, Weinfeld & Paigen, 1967).

Some of the conditions causing loss of restriction are also known to cause disturbances of the bacterial periplasm, a fact that argues for the location of the restriction enzyme near to the surface membrane of the bacterium (Fukasawa, 1964b; Molholt & Fraser, 1965; Schell & Glover, 1965, 1966a,b).

ii The fate of the restricted DNA

Lederberg (1957), while demonstrating that the injection of ^{32}P-labelled DNA of restricted T1 into B(P1) was normal, also observed that the ^{32}P could be washed out of the cells. The most detailed study of the degradation of ^{32}P-labelled DNA to acid-soluble fragments was carried out by Dussoix & Arber (1962) using λ.K in K(P1). The release of acid-soluble ^{32}P from the cells commenced within a few minutes of infection, and reached a plateau, equivalent to as much as 80% of the ^{32}P, in 10 to 20 min. Initially most of the acid soluble counts were associated with the bacteria, and more acid-soluble ^{32}P was found if the cells were gently lysed prior to acid treatment. Eventually, the majority of acid-soluble ^{32}P was in the medium and no longer associated with the cells, though there was always a fraction of the ^{32}P which was never released and was thought to represent material reutilised by the bacteria. It was concluded from such experiments that the acid-solubilisation of the ^{32}P, and therefore the

breakdown of the restricted DNA, was an intracellular event, and that the breakdown proceeded until all the DNA was acid soluble. What the experiment failed to show is whether the degradation of the DNA was essential to the restriction process, or merely a consequence of it.

There are several lines of evidence that suggest that the restricted DNA is initially present in the cell in "gene-sized" or larger fragments. Paigen & Weinfeld (1963), extending an earlier observation of Lederberg (1957) on T1 restriction by B(P1), found in the restriction of phage λ.C by K an increase in successful infections with multiplicity of infecting phage which could be explained by a cooperation of restricted phage genomes at high multiplicities of infection. Marker rescue experiments imply the survival of fragments of phage genome large enough for recombination to occur, or for a copy-choice mechanism to operate. The efficiency of marker rescue is a time dependent process, decreasing with an increase in the interval between infection with a restricted phage and with the rescuing phage (Luria & Human, 1952; Garen & Zinder, 1955; Drexler & Christensen, 1961; Christensen, 1962; Dussoix & Arber, 1962; Terzi, 1968). Dussoix & Arber (1962) suggested that the time dependence of rescue reflected the slower degradation of DNA fragments after the initial restriction.

Complementation which involves a restricted phage implies expression of the restricted phage genes. Terzi (1968) developed a system to minimise marker rescue, which had obscured the results of previous attempts to demonstrate complementation. He used a K host which was recombination deficient (Rec^-), as well as being Su^-. The infecting phages carried two different sus mutations, one on a phage

both lacking the λ recombinase (cI⁻red⁻)

λ.C, and the other on a phage λ.K. Pure bursts of phage λsus.K parental type were obtained from these experiments, indicating that the function lacked by the phage λ.K must have been supplied by the restricted phage λ.C. The fact that recombinant progeny phages were ten fold fewer in this experiment than when the phage λ carried an intact recombinase strongly suggests that the residual production of parental type phage λsus.K was due to complementation.

The other type of experiment where survival of restricted phage genes has been demonstrated involves the direct measurement of phage specific enzymes in the restricting host. This has been performed for T*2 and T2gt (Hattman et al., 1966).

iii In vitro restriction

Results of in vitro restriction experiments using crude extracts gave ambiguous, though encouraging, results (Fukasawa, 1964b; Takano, Watanabe & Fukasawa, 1966). Linn & Arber (1968) utilised the observation of Benzinger (1968) that the replicative form (RF) of phage fd is infectious for Escherichia coli spheroplasts, and the spheroplasts of B and P1 lysogens retain the ability to restrict. A purified extract of B caused loss of infectivity of fd RF.C, but not of fd RF.B, on incubation. A similar enzyme from B(P1) attacked fd RF.B but not fd RF.B(P1). No enzyme causing loss of infectivity of fd RF.C was isolated from C, K or restrictionless mutants of B. On the basis of electron micrographs, it was suggested that the breakdown observed was not extensive.

The elegant experiments of Meselson & Yuan (1968) not only demonstrated the isolation of a restriction enzyme, but also supplied information on the course of the reaction, and analysed the reaction products. In these experiments, the ^{32}P-labelled phage λ.C was

was incubated with the extract, and then the analysis of the products was performed by centrifugation on a density gradient on which a control tritium-labelled phage λ.K DNA was also included. If incubation was executed in the absence of purified extract, or if the extract came from a restrictionless mutant of K, the DNA from phage λ.C and λ$\frac{1}{2}$K banded together on the gradient. But when the enzyme had been prepared from K, the ^{32}P-labelled material had a decreased sedimentation rate. The ^{32}P material from phage λ.C DNA did not appear as non-sedimenting small molecules, but as molecules of DNA about one quarter the size of a phage λ chromosome. It was confirmed that the enzyme is an endonuclease by using as substrate for the incubation twisted circles of phage λ DNA. Since these are entirely covalently bonded molecules, there is no free end for an exonuclease to attack. Not only were the twisted circles attacked, but by using a very short incubation time the presence of untwisted circles was detected by a change in bouyant density on the density gradient. This result clearly indicates that the restriction process involves breakage of one strand of the DNA followed by breakage of the second strand (appearance of linear molecules on the density gradient). Since the enzyme did not attack heteroduplex DNA molecules, where one strand carried modification and the other did not, it can also be concluded that the enzyme recognises the duplex, but is active on only one strand of it at a time. The requirements of the enzyme which have been so far identified are ATP, Mg^{++}, and SAM, this last may have implications concerning the mode of action of the enzyme, but it is not yet known if it is merely an activator or a substrate.

E. The Genetics of Host Controlled Modification

i The Escherichia coli Chromosomal host specificity

The most detailed genetic information is available for the Escherichia coli chromosomal genes determining the host specificities of types K, B, and to a lesser extent, 15, the specificity carried by Escherichia coli 15. Analysis of recombinants from Hfr crosses and phage P1 transductions located the host specificity genes to the right of, and close to, threonine; the K, B and 15 host specificity genes were also shown to be allelic (Boyer, 1964; Colson, Glover, Symonds & Stacey, 1965; Hoekstra & De Haan, 1965; Lederberg, 1966; Wood, 1966). The location of these genes was defined more precisely when they were shown to be 20% cotransduced with serB (Glover & Colson, 1969). There is no fine structure mapping of these genes, even the gross structural arrangement of genes in the host specificity region is difficult to elucidate if only conjugation and transduction techniques are used. Wild type recombinants have been obtained in transductions involving two different mutants (Glover & Colson, 1969), but lack of selection procedure for a modification phenotype (m), and practical problems in scoring the restriction phenotype (r), have made the mapping of this region of the chromosome difficult.

Even though there is still no order for the host specificity genes on the chromosome, information on the number and function of these genes has accumulated, stemming from the isolation of restrictionless (r^-) mutants. Several reasonably efficient selection procedures have been developed for the isolation of restrictionless mutants (Colson et al., 1965; Wood, 1965; Lederberg, 1966). Two classes of restrictionless mutant were isolated; one of these retained the ability to modify and hence had an r^-m^+

phenotype; the other did not modify and was phenotypically r^-m^-. The two mutant classes were found with both B and K, and were equally common (Colson *et al.*, 1965; Wood, 1965, 1966; Lederberg, 1966).

The existence of two phenotypes was a strong indication that a minimum of two genes were involved in host specificity. These data have now been supplemented by complementation studies where partially diploid cells are made by infecting with F-prime factors carrying the host specificity genes. The two mutations described, the r^-m^- and the r^-m^+ complemented each other, the partial diploid having an r^+ phenotype. The lesions causing the r^-m^- and r^-m^+ phenotypes must be in different genes, unless intragenic complementation is occurring, which total restoration of restriction made unlikely. The gene in which a mutation produces the r^-m^+ phenotype is referred to as *hsr*, while it is a mutation in the gene termed *hss* which causes the so-called single-step r^-m^- phenotype. The existence of a third gene, *hsm*, affecting only modification, was demonstrated by complementation using the so-called two-step r^-m^- mutants, obtained by selecting m^- mutants from r^-m^+. If the two-step mutant had the second lesion in the *hss* gene, so that it was genotypically hss^-hsr^-, it could not complement either the single-step r^-m^-(hss^-) or the r^-m^+ (hsr^-) mutants. Such a class of two-step r^-m^- mutants was found. But a second class of two-step mutant which complemented the r^-m^- (hss^-), restoring both restriction and modification, was also found. This second class of two-step mutant restored modification to the other two-step mutant (hss^-hsr^-). Clearly this second class of two-step mutant could be designated $hss^+hsr^-hsm^-$, all other classes of mutant being hsm^+. Not only did the same scheme apply to the

mutants of K and B, but they complemented each other. For example, an r^-m^+ B complemented an r^-m^-(*hss*$^-$) K, though the restriction and modification were of the B type only (specificity resides in the *hss* gene, chap. 1 section I.F). (Glover unpublished results; Boyer & Roulland-Dussoix, 1969).

Proof is still lacking that the host specificity genes are contiguous on the *Escherichia coli* chromosome, though they must lie very close together, since all mutations so far tested are cotransduced with *ser*B by P1 (Glover & Colson, 1969; Boyer & Roulland-Dussoix, 1969). The existence of a fourth gene has been suggested by Boyer & Roulland-Dussoix (1969), since they observed a trans dominant r^- mutation not previously reported. There is no evidence for an operon structure, or for any form of regulation, unless partially restricting and modifying mutants are due to regulatory misfunction, as suggested by Boyer & Roulland-Dussoix (1969).

ii Genetics of other systems

The genetic control of restriction of the T*-even phages differs profoundly from that discussed in chap. 1 section I.E.i, and from the P1 controlled host specificity, where the mutations observed are of the same phenotypes as seen for the chromosomal host specificity of *Escherichia coli* (Glover, Schell, Symonds, & Stacey, 1963). The restriction of the T*-even phages is a function totally separate from the modification (discussed in chap. 1 section I.E.ii); the genetic determinants are likewise separate. Mutants to permissiveness in B are *thi*$^-$, and absence of revertants suggests that they are deletions. In K two phenotypes are seen, permissiveness to T*6, and permissiveness to T*-even phages, due to two possible types of mutation, one confering the permissivenss to phage T*6, the other to T*2 and T*4 phages, but only expressed in the presence of the former mutation.

The Salmonella host specificity, determining restriction and modification of phage P22, maps in the region of lac and T6 resistance. (Zinder, 1960; Lederberg, 1966; Colson & Colson, 1967). Colson & Colson (1967) has isolated mutants with r^-m^- and r^-m^+ phenotypes, so that there is at least a resemblance to the Escherichia coli host specificity.

F. Specificity in the Restriction and Modification Processes

Host specificity is not merely observed for a few bacteriophages of Escherichia coli. In E. coli itself, not only are many phages subject to host specificity (Eskridge et al., 1967) but bacterial chromosomal and plasmid DNA is also restricted and modified (Arber, 1962; Boice & Luria, 1963; De Haan, Stouthamer, Felix & Mol, 1963; Glover et al., 1963; Boyer, 1964; Pittard, 1964; Arber & Morse, 1965; Hoekstra & De Haan, 1965).

The phage λ chromosome probably only has a limited number of sites at which the restriction and modification enzymes can act, the main evidence for this is the limited breakdown of restricted phage λ DNA by the K specific restriction enzyme (chap. 1 section I.D.iii). Irrespective of the number of sites, it is clear that the restriction and modification enzymes of a particular specificity must recognise the same sites on the DNA, since it is modification of the site which protects the DNA from the restriction enzyme. This concept, coupled with the existence of single mutations giving the r^-m^- phenotype (chap. 1 section I.E.i) has lead to the idea that the restriction and modification enzymes are oligomers, each composed of at least two types of subunit. One subunit, product of the hss gene (chap. 1 section I.E.i) would be common to both enzymes and would determine specificity. Support for this model may become available

from enzyme studies, which have already suggested that the K restriction enzyme is an oligomer (Meselson & Yuan, 1968).

It is assumed that both enzymes recognise a finite base sequence on the DNA. It is not yet known what this base sequence is for any enzyme. If a recognition site could be located in a gene with a known protein product, specific mutagenesis might allow identification of necessary bases in the sequence. Unfortunately, location of recognition sites on phages is hindered by the fact that most phages carry several such sites. Those phages, which, like fd, carry only one or two sites, have been little used for genetic mapping, so that there would be less chance that a protein product would be known for a gene in which a site might occur. Arber (1969) has mapped a mutation on phage λ which rendered it free from the restriction of type A (a new type of host specificity found in recombinants of 15 and K). The phage was apparently normal, but the mutation mapped between the genes O and CII, and would therefore be within a transcription unit. Indirectly, the experiments of Terzi (1968) by failing to show any complementation for markers to the left of N on the phage λ chromosome suggested a K specific site near to N.

An elegant approach to the problems both of mapping and of base sequence identification is now available as a result of the study of hybrids of phages λ and ø80. Phage ø80 has an e.o.p. on K so much lower than that of phage λ that it was possible that the phage ø80 chromosome might only carry only one K recognition site. Certain hybrid λ-ø80 phages were isolated, one of which had an e.o.p. of only 10^{-2} on K. This hybrid had the right arm of phage λ, including the red gene, and the left arm of phage ø80 including the att ø80 (Franklin & Dove, 1969). Mutants of this hybrid phage not restricted

on K have been obtained without any obvious loss of function, and the site of the mutation located near to N. It is hoped to use phage λ-ø80 hybrids containing separately the various K specific recognition sites of phage λ, coupled with use of the K restriction enzyme and techniques developed for the sequencing of short segments of DNA to investigate the restriction sites of phage λ (N. Murray, personal communication). Extension of this method could lead to sequencing of DNA, depending on the availability of restriction enzymes and phage mutants lacking all but one restriction site.

SECTION II

TRANSMISSIBLE DRUG RESISTANCE FACTORS IN THE ENTEROBACTERIACAEA

A. Incidence and Nature of Multiple Drug Resistance

The term transmissible drug resistance factor (R factor) has been applied to any plasmid which determines resistance to an antibiotic and will transfer this drug resistance to a sensitive strain. The first R factor isolated determined resistance to four antibiotics in common use, Tetracycline (Tc), Streptomycin (Sm), Sulphonamide (Su) and Chloramphenicol (Cm) (Kitamoto, Kasai, Fukaya & Kawashma, 1956). R factors have also been isolated which determine resistance to penicillins (Anderson & Datta, 1965), Kanamycin (Km) and Neomycin (Lebek, 1963). An R factor may carry resistance to only one drug, or to several (Lebek, 1969). In the few cases studied, R factors have been found to determine the production of an enzyme which inactivates the drug to which they cause resistance. Certainly R factors which determine resistance to penicillin also determine the production of a penicillinase (Datta & Kontamichalou, 1965; Datta & Richmond, 1966; Egawa, Sawai & Mitsuhashi, 1967); acetylating enzymes have been observed for streptomycin and chloramphenicol (Okamoto & Suzuki, 1965), and an adenylating enzyme has been found for streptomycin (Yamada, Tipper & Davies, 1968). It is still a possibility that drug resistance may in other cases be due to changes in permeability of the cell, as suggested by Unowsky & Rachmeler (1966).

With regard to the molecular nature of the R factors, their resemblance to other sex factors (see chap. 1 section II.B) suggests that they are DNA molecules, and inactivation of the R factor by the decay of incorporated ^{32}P likewise indicates a nucleic acid composition.

The most convincing evidence that R factors are DNA molecules has come from the use of Proteus mirabilis to which R factors have been successfully transferred. Density gradient centrifugation of extracts of Proteus mirabilis carrying an R factor showed a new band of DNA not present in uninfected Proteus mirabilis extracts. The difference in bouyant density between the Proteus mirabilis chromosomal DNA and the R factor DNA was sufficient to allow the isolation of the R factor DNA (Falkow, Citarella, Wolheiter & Watanabe, 1966). Electron micrographs of isolated R factor DNA have demonstrated the existence of a circular form (Nisioka, Mitani & Clowes, 1969).

B. Transfer of Drug Resistance

The transmissible nature of the multiple drug resistance was first detected when, after mixed cultivation of a drug resistant and a sensitive strain, resistant bacteria with the genotype of the initially sensitive strain were isolated. Mitsuhashi, Harada & Hashimoto (1960a), showed that the conversion of the sensitive cell to drug resistance was not brought about by a bacteriophage or any other filtrable agent. The dependence of transfer upon cell contact between resistant and sensitive strains was demonstrated by Watanabe & Fukasawa (1961a).

In the Enterobacteriaceae, one method of transmission of genetic information is known which is dependent on cell to cell contact. This method of genetic transfer depends on the presence in the donor cell of a sex factor carrying genes determining the functions necessary for cell to cell contact and for transfer of genetic information between the cells. The first sex-factor to be identified was discovered in a strain of Escherichia coli K12, and was termed F

(Lederberg, Cavalli & Lederberg, 1952). The sex-factor F can exist in an autonomous state in the cytoplasm of the host cell, which is therefore termed an F⁺ cell, in this location it promotes its own transfer. The F factor can also form a stable association with the Escherichia coli chromosome, by integration, to produce a cell, termed an Hfr, which can transfer chromosomal genes to recipient strains. Hfr strains transfer the chromosome in an orientated fashion from the point of insertion of F, and transfer is unidirectional (Wollman & Jacob, 1955, 1958). The F factor will also increase the frequency of transfer of plasmids, like Col E1, which have a very low rate of transfer in the absence of a sex factor (Clowes, 1965).

Transfer of drug resistance was found to be independent of the presence of F or any other known plasmid in the donor cell (Mitsuhashi, Harada, Kameda, Suzuki & Egawa, 1960). It was therefore concluded that the genes determining resistance to antibiotics (resistance determinants) are attached to, or are part of, a sex factor. The term R factor will be used in this thesis to refer to the combination of sex factor and resistance determinants. The evidence available points to a cytoplasmic, as opposed to chromosomal, location for these genetic elements. Spontaneous loss of R factors has occurred (Mitsuhashi, Harada & Kameda, 1961a) and elimination of R factors by treatments believed to act via differential effects on cytoplasmic and chromosomal genes has also been observed (Mitsuhashi et al., 1961a, b; Tomoeda, Inuzuka, Kubo & Nakamura, 1968; Bouanchaud, Scavizzi & Chabbert, 1969). It is particularly interesting that some R factors are eliminated by acridine dyes (Mitsuhashi et al., 1961a, b) since this is a method

that has been used for the effective curing of F^+ cells (Hirota, 1960). While R factors clearly have a cytoplasmic location, R^+ cultures have been found to transfer chromosomal markers, though at a low frequency compared with F^+ cultures (Sugino & Hirota, 1962). An R factor which shows a marked preference for association with a particular region of the chromosome has been studied, and the transfer of chromosomal genes from this strain resembled transfer from an Hfr strain (Pearce & Meynell, 1968). The authors, however, suggested that the behaviour of this R^+ strain resembles that of strains carrying F' factors such as the F2 factor (Adelberg & Burns, 1960) in that recombinants for chromosomal genes are normally R^+, while with an Hfr donor recombinants are generally F^-.

While the similarity between F and R support the concept that R factors are sex factors, there are also some striking differences between them. Transfer of drug resistance is much lower than transfer of F, and transfer of chromosomal genes by R factors occurs at a much lower frequency than when transfer is F-mediated (Sugino & Hirota, 1962). R^+ cultures are not lysed macroscopically by F specific phages which adsorb to the F pilus and lyse F^+ cells (Brinton, Gemski & Carnahan, 1964; Crawford & Gesteland, 1964; Hoffman-Berling & Maze, 1964; Caro & Schnos, 1966), and no stable integration of the R factor into the chromosome has been reported.

The R factors show resemblance to some colicin factors, such as Col I which, while promoting its own transfer does so at a much lower frequency than F. Another point of similarity between Col I and R factors is the production of a high frequency transfer (HFT) system following the transfer of either Col I or an R factor to a new host (Stocker, Smith & Ozeki, 1963; Watanabe, 1963). Most of the

bacteria that have received a Col I or an R factor are capable of transfer, but after a few generations the transfer frequency returns to the low value of the established culture. The explanation of this observation is thought to lie in the repression of the functions involved in the transfer of Col I or an R factor. In the established culture, the functions are repressed and transfer is low, but when the sex factor is transferred to a new host there is no cytoplasmic repressor present, the sex factor expresses the necessary genes for transfer, and the transfer frequency of the culture as a whole is higher than in the established culture. Accumulation of repressed cells leads to a decline in the frequency of transfer. This theory received support from the discovery that some R factors repress the expression of F in cells carrying both plasmids (Watanabe & Fukasawa, 1962). Not only is F-mediated conjugation reduced in these strains, but macroscopic lysis by F specific phages no longer occurs (Watanabe & Fukasawa, 1962) and the culture is not aggluctinated by F-specific antisera (Hirota, Nishimura, Ørskov & Ørskov, 1964). R factors which display this suppression of the functions of F (fertility inhibition) are termed fi$^+$. There is a second class of R factor, which does not repress F, and these are termed fi$^-$ (Watanabe, Nishida, Ogata, Arai & Saito, 1964a). Meynell & Datta (1966a) first suggested that the repression of F by fi$^+$ R factors was an expression of the close phylogenetic relationship between these two factors since the F genes respond to the R factor repressor. Proof of the suggestion rests on the isolation of the predicted derepressed R factors by Meynell & Datta (1967). This work showed that the fi$^+$ R factor is normally repressed since the derepressed mutants now produce pili on the majority of cells, conjugate and transfer the R factor at

a much higher frequency than the repressed factor, and are macroscopically lysed by F specific phage.

The isolation of these derepressed mutants also aided the demonstration that the pili produced by fi⁺ R factors are closely related to F pili. The F pilus is the site of attachment of F specific phages, and while it was demonstrated that cultures of fi⁺ R factors, particularly HFT cultures, will increase the titre of F specific phage (Meynell & Datta, 1965, 1966), it was advantageous to use derepressed mutants to show adsorption of F specific phage to the R factor pilus in electron micrographs. It is similarly possible to show adsorption of F-specific antisera to the R pilus (Nishimura, Ishibashi, Meynell & Hirota, 1967; Lawn, Meynell, Meynell & Datta, 1967). It has been found that the derepressed phenotype is recessive in cells carrying both mutant and wild type fi⁺ R factors (Meynell & Datta, 1967); this implies a defect in repressor activity, not in the operator.

The low frequency of transfer of fi⁻ R factors, and the production of HFT systems with them, made it unlikely that they were repressor (i⁻) mutants of fi⁺ R factors. As with fi⁺ R factors, derepressed mutants of fi⁻ R factors have been isolated (Meynell & Datta, 1967), showing that fi⁻ R factors are also repressed sex factors. The F operator is not sensitive to the fi⁻ R factor repressor, indicating that fi⁻ R factors lack the relationship with F seen with the fi⁺ R factors. However, the isolation of a new type of filamentous phage, adsorbing specifically to the pili produced by derepressed fi⁻ R factors (Meynell & Lawn, 1968), revealed a relationship with other sex factors. The newly isolated phage, termed If1, adsorbs both to the pili produced by fi⁻ R factors and to those produced by Col I. And similarly antisera to Col I adsorbs to the

fi⁻ R factor pilus (Lawn et al., 1967). Derepressed mutants of Col I have now been obtained, and it seems that a majority, if not all, sex factors found in the Enterobacteriaceae may belong to one or other of the two types, the F-like and the I-like (Meynell, Meynell & Datta, 1968).

C. Superinfection Immunity

The term superinfection immunity is used for the exclusion and/or suppression of multiplication of a plasmid in a strain carrying an other plasmid. The term arose in relation to phage infected bacteria, which may show superinfection immunity if infected with a second phage; but the phenomenon is also observed between sex factors. F⁺ cells display superinfection immunity towards other F factors transferred to them (Lederberg et al., 1962; Scaife & Gross, 1962). In contrast, the presence of F in a bacterial strain does not significantly alter the ability of the strain to act as a recipient for R factors, the difference in transfer frequency to an R⁻ and an R⁺ culture is only about five fold at most (Mitsuhashi, Harada, Kameda, Suzuki & Egawa, 1960b; Watanabe & Fukasawa, 1961a, 1962). While on the one hand a cell may carry both an fi⁻ and an fi⁺ R factor, and on the other hand F will coexist stably with either type of R factor, it was found that the presence of two fi⁻, or two fi⁺, R factors in the same cell was not a stable combination (Harada, Kameda, Suzuki, Kakinuma & Mitsuhashi, 1961; Mitsuhashi, Harada, Hashimoto, Kameda & Suzuki, 1962; Watanabe et al., 1964a). The two R factors may be segregants from a single parent R factor, or independent isolates, but in either case the frequency of transfer of one R factor to a cell containing the other is reduced (Watanabe & Fukasawa, 1962; Molina, Schito, Calegri & Romanzi, 1965). Forced

co-existence of two R factors of the same fi type is possible if they are maintained on media selective for the presence of both factors (Harada et al., 1961; Mitsuhashi et al., 1962; Watanabe et al., 1964a), but separation into the individual R factors occurs if the strain is subcultured on media lacking drugs. Recombinants, carrying all the drug resistances of the original R factors can be isolated after a period of enforced joint maintenance (Mitsuhashi et al., 1962; Watanabe et al., 1964a). These recombinant R factors appear to be true recombinants, existing as a single structural unit, since the resistance determinants and the sex factor are transduced as one entity by phage P1 (chap. 1 section II.D) (Mitsuhashi et al., 1962; Watanabe et al., 1964a).

D. Transduction of R Factors

The resistance determinants, and in some instances, the associated sex factor, can be transduced. In Escherichia coli joint transduction by phage P1 of the resistance determinants and sex factor occurs (Watanabe & Fukasawa, 1961b; Kondo, Harada & Mitsuhashi, 1962) and it is argued that this will only occur if the R factor is one continuous DNA molecule. Phage P1 transduction has therefore been used to demonstrate the presence of only one R factor in presumptive recombinants between two R factors (chap. 1 section II.C) and to show the occurrence of recombination between F and an R factor (Watanabe & Fukasawa, 1961b; Mitsuhashi et al., 1962; Harada, Kameda, Suzuki & Mitsuhashi, 1964; Watanabe et al., 1964a).

Transduction of R factors in Salmonella has been observed with phages P22, epsilon 15 and epsilon 34. The major difference between transductions with these phages and with phage P1 is the rarity with which the entire R factor is transduced by the Salmonella phages.

It has been suggested that in the case of P22, this may simply be a reflection of the fact that phage P22 normally transduces shorter segments of DNA than phage P1 (Watanabe & Fukasawa, 1961b). However, Dubnau & Stocker (1964) isolated phage P22 transduced strains which they believed to contain recombinants of the R factor and phage P22, the recombinant factor now being integrated into the chromosome. Similar results have also been reported with the epsilon phages (Harada, Kameda, Suzuki & Mitsuhashi, 1963).

B. Association of Other Genetic Determinants with R Factors

R factors have been classified together as a group of sex factors purely on the basis of the transmissibility of the associated resistance determinants. Meynell et al.,(1968) pointed out that this was not necessarily a meaningful exercise, placing these sex factors in a class distinct from all other sex factors. On the contrary, the R factors were clearly internally subdivided into fi$^+$ and fi$^-$ (chap. 1 section II.C) and the two types were closely related in some functions, such as type of pilus produced, to other sex factors. It is not therefore surprising that the R factors not only have resistance determinants associated with the sex factor, but also other genetic determinants as well. Colicin production and resistance were found associated with R factors by Siccardi (1966). Determinants have been reported which affect the plating of phages on R$^+$ strains, those R$^+$ strains, observed by Siccardi (1966), which were resistant to Col Ib also reduce the e.o.p. of the phages BF23 and W31. Other examples show that R factors can carry determinants for the restriction and modification of phages. In Salmonella for example, the phage type of strain is partly determined by the

the presence of genes restricting and modifying the typing phages. An R factor has been shown to alter the phage type of Salmonella in the same manner as lysogenisation with a temperate phage (Anderson & Lewis, 1965). Guinee & Willems (1967) found not only restriction and modification of a Salmonella phage, but also found a reduction in e.o.p. of phage λ to be associated with some R factors. Restriction and modification of coli phages by R factors has been demonstrated for an R factor initially isolated in Klebsiella pneumoniae (Molina, 1964), and for R factors isolated from Escherichia coli and Shigella (Yoshikawa & Akiba, 1962; Watanabe, Nishida, Arai, Sato & Ogata, 1964b; Watanabe, Takano, Arai, Nishida & Saito, 1966).

CHAPTER 2

MATERIALS AND METHODS

A. Media

Oxoid no. 2 broth: Oxoid no. 2 broth powder 25g; distilled water 1 l.

L-Broth: Difco tryptone 10g; yeast extract 5g; NaCl 10g; distilled water 1 l. pH 7.2

Phage broth: Difco Bacto peptone 15g; Difco Bacto nutrient broth 8g; NaCl 8g; glucose 1g; distilled water 1 l. pH 7.2

EMB nutrient base: Difco Bacto casamino acids 42.4g; Difco Bacto yeast extract 5.2g; NaCl 27g; K_2HPO_4 10.4g; distilled water 1 l.

VB salts (X20): $MgSO_4.7H_2O$ 6g; K_2HPO_4 300g; $NaNH_4HPO_4.4H_2O$ 105g; citric acid.$1H_2O$ 60g; distilled water 1,332 ml.

M9 salts (X4): Na_2HPO_4 28g; KH_2PO_4 12g; NaCl 2g; NH_4Cl 4g; distilled water 1 l.

M9 minimal medium: M9 salts (X4) diluted 1/4 in distilled water, with addition of $MgSO_4$ to 0.001 M and glucose to 0.2%.

M9 Buffer: M9 salts (X4) diluted 1/4 in distilled water, with addition of $MgSO_4$ to 0.001 M, and $CaCl_2$ to 0.0001 M.

Phage Buffer: KH_2PO_4 3g; Na_2HPO_4 7g; NaCl 5g; $MgSO_4$ (0.1 M solution) 10 ml; $CaCl_2$ (0.001 M solution) 10 ml; gelatin solution (1%) 1 ml; distilled water 1 l.

Buffer (Bacterial): KH_2PO_4 3g; Na_2HPO_4 7g; NaCl 4g; $MgSO_4.7H_2O$ 0.2g; distilled water 1 l.

λ adsorption medium: 0.01 M $MgSO_4$ solution.

Oxoid nutrient agar: Oxoid no. 2 broth solidified with 12.5g/l of Davis New Zealand agar.

Difco agar: Oxoid tryptone 10g; NaCl 8g; glucose 1g; Difco Bacto agar 10g; distilled water 1 l.

Water agar: Davis New Zealand agar 20g; distilled water 1 l. pH 7.2.

VB agar: VB salts (X20) were added to water agar and distilled water so that the final agar concentration was 1.5%.

Water soft agar: Difco Bacto agar 6g; distilled water 1 l.

Difco stab agar: Oxoid tryptone 10g; NaCl 7g; glucose 3g; Difco Bacto agar 10g; distilled water 1 l.

EMB dyes: Eosin yellow 4% (w/v); methylene blue 0.65% (w/v).

EMB agar: 300 ml water agar; 4 ml eosin solution; 4 ml methylene blue solution; 75 ml nutrient base; 20 ml of a 20% sugar solution.

Amino Acids: Made as a solution of 2 mg/ml and used at a final concentration of 20 µg/ml.

Vitamin B_1 (thiamine hydrochloride): Used at a final concentration of 1 µg/ml.

Thymine: Used at a final concentration of 40 µg/ml.

Sugars: Made as 20% solutions and used at a final concentration of 0.2%, except in EMB agar, where the final concentration is 1%.

Penicillin: Benzyl penicillin (Glaxo) used at a final concentration of 20 µg/ml.

Tetracycline: Achromycin hydrochloride (Cyanamid) used at a final concentration of 50 µg/ml.

Streptomycin: Streptomycin sulphate BP (Glaxo) used at a final concentration of 200 µg/ml, for contraselection with chromosomal markers, and at a final concentration of 20 µg/ml. for selection for R factor resistance.

Sulphonamide: Sulphamezathine (ICI) used at a final concentration of 20 µg/ml.

Kanamycin: Kanamycin sulphate BPC (Bayer) used at a final concentration of 20 µg/ml.

Chloramphenicol: Chloromycetin (Parke Davis) used at a final concentration of 20 µg/ml (diluted from a 1000X solution in methanol).

Multodisks: Oxoid Multodisks code no. 30-19N, carrying the following drugs impregnated on the paper:- tetracycline, streptomycin, sulphonamide, kanamycin, chloramphenicol, ampicillin.

B. Bacteria

The strains of Escherichia coli used are listed in Table 2.1. Bacterial strains in use were maintained on Oxoid nutrient agar plates at 4^{o}. R^{+} strains were maintained on appropriately supplemented VB agar plates, containing one of the drugs to which the strain was resistant, also at 4^{o}. All strains were maintained on Difco agar stabs at room temperature.

C. R Factors

The J5-3 R^{+} strains used in the initial survey were the gift of Dr. Naomi Datta and Dr. Elinor Meynell. The original strains have not been listed, but the R factors corresponding to the R^{+} strains assigned to groups I to X (chap. 3) are listed in Table 2.2. These are followed by a list of the remaining R factors corresponding to

Table 2.1.

Bacterial Strains

Strain designation	Characteristics	Source	Reference
C600	thr⁻leu⁻thi⁻lac⁻hsK	S. W. Glover	Appleyard, 1959
C600(P1)	thr⁻leu⁻thi⁻lac⁻hsK(P1)	S. W. Glover	
C600(λ CI_{857})	thr⁻leu⁻thi⁻lac⁻hsK (λ CI_{857})	S. W. Glover	
J5-3	pro⁻met⁻hsK (λ)	E. Meynell	Clowes & Rowley, 1954
J5-3 F^+	pro⁻met⁻hsK (λ) F^+	E. Meynell	
E7110	F' pro^+lac^+/pro⁻lac⁻ara⁻thi⁻hsK	J. Scaife	
4K	thr⁻leu⁻thi⁻serB⁻lac⁻r⁻m⁻K	S. W. Glover	
7K	thr⁻leu⁻thi⁻hsK	S. W. Glover	
15	thi⁻thy⁻hs15	K. A. Stacey	Stacey, 1965
C	prototroph	S. W. Glover	Bertani & Weigle, 1953
C T⁻	thr⁻	S. W. Glover	
B251	prototroph hsB	S. W. Glover	Arber & Lataste-Dorolle, 1961
B trp⁻	trp⁻hsB	S. W. Glover	
B6	thr⁻leu⁻ile⁻ r^-m^+B	S. W. Glover	
B8	thr⁻leu⁻ile⁻lac⁻ r^-m^-B	S. W. Glover	
Ib^+	met⁻ $\lambda^R colIb^+ colI^R$	E. Moody	

Table 2.2.

The R factors of the original J5-R+ Strains

delegated to one of the groups I to X

R factor	Resistances	fi*	colI** production	colIb*** resistance	Group
R39	Tc	-	+	R	IX
R41	Tc Sm	-	-	S	V
R56	Tc Su	-	-	S	V
R62	Tc Sm	+/-	+	R	IX
R64	Tc Sm	-	-	S	V
R70	Sm Su		-	S	X
R73	Tc Sm Su	+/-	-	S	X
R75	Tc Sm Su		-	S	X
R77	Tc Sm Su	+/-	-	S	X
R124	Tc	+	-	S	I
R132	Tc Sm Su	-	-	S	II
R143	Km	-	+	R	IX
R144	Km	-	+	R	IX
R145	Km	-	+	R	IX
R163	Km	-	+	R	IX
R191	Tc Sm Su Cm	+	-	S	VII
R193	Tc Sm Su Cm	+	+	R	IX
R194	Tc Sm	+	+	R	IX
R196	Tc Sm Su Cm	+/-	-	-	VII
R199	Tc	-	-	S	II
R203	Tc	-	-	S	II
R204	Tc	-	-	S	II

Table 2.2. (Cont'd)

R factor	Resistances	fi*	colI** production	colIb*** resistance	Group
R230	Sm Su	+	-	S	III
R231	Tc Sm Su Cm	+	-	S	III
R234	Tc Sm Su Cm	+	-	S	III
R241	Tc Su	-	+	R	IX
R243	Tc Sm Su Cm	+	-	S	X
R245	Tc	-	-	S	II
R250	Tc Su	-	-	S	II
R252	Su	-	-	R	VI
R265	Tc	-	-	S	II
R269	Tc Sm Su Km	+/-	-	S	II
R270	Tc Sm Su	-	-	S	II
R276	Tc Sm Su Km Cm	+	-	S	IV
R295	Sm Su	-			X
R296	Sm Su	-			X
R297	Sm Su	-			X
R298	Sm Su	-			X
R299	Sm Su	-			X
R300	Sm Su	-			X
R301	Sm Su	+			X
R302	Sm Su	-			X
R303	Sm Su	-			X
R304	Sm Su	-			X
R305	Sm Su	-			X
R306	Sm Su	-			X
R307	Sm Su	-			X

Table 2.2. (Cont'd)

R factor	Resistance	fi*	colI** production	colIb*** resistance	Group
R309	Sm Su	-			X
R310	Sm Su	-			X
R311	Sm Su	-			X
R313	Tc Sm Su	-	-	S	II
R318	Sm Su Km	+	-	S	VIII
R324	Su Km	-	+	R	IX
R329	Tc Sm Su Cm	+	-	S	III
R330	Sm Su Km	-	-	S	IX
R336	Sm Su	-			X
R337	Sm Su	-			X
R338	Sm Su	-			X
R340	Sm Su	-	-	S	VIII
R341	Sm Su	-	-	S	VIII
R348	Tc Sm Km Cm	+/-	-	S	II

* fi character is that scored by Drs. Datta & Meynell, with exceptions which will be commented on in the text. The sign +/- indicates that the original strain contained an fi^- and fi^+ R factor.

** Colicin I production recorded here is based on tests as detailed in chap. 2 section Q.i, + indicates production, - indicates no production.

*** Colicin Ib resistance recorded here is based on tests by the method of chap. 2 section Q.ii, R indicates resistance, S indicates sensitivity.

Appendix to Table 2.2.

The following are a list of the numbers given to the R factors in the R^+ strains which were not found to reduce the e.o.p. of any of the phages used in this survey:-

R1, R3, R4, R5, R6, R7, R8, R9, R11, R12, R13, R14, R15, R17, R18, R36, R45, R46, R51, R52, R82, R92, R114, R136, R142, R192, R195, R198, R200, R201, R215, R217, R218, R219, R221, R222, R224, R225, R227, R228, R229, R237, R242, R253, R255, R257, R258, R262, R263, R265, R266, R267, R271, R272, R273, R274, R275, R277, R281, R283, R284, R285, R286, R287, R289, R290, R291, R292, R293, R294, R312, R314, R315, R316, R317, R319, R320, R321, R322, R323, R325, R326, R327, R328, R331, R334, R335, R339, R342, R343, R344.

Table 2.3.

Segregant R factors obtained

from strains of groups I & II

R factor	Resistances	Host specificity	fi*	Method obtained
R132-SS-1	Sm Su	II	(-)	conjugation
R269-SSK-1	Sm Su Km		+	conjugation
R269-TSS-1	Tc Sm Su	II	-	conjugation
R313-T-1	Tc	II	-	penicillin selection
R313-T-2	Tc	II	(-)	P1 transduction
R313-T-3	Tc	II	(-)	conjugation
R313-T-4	Tc	II	(-)	P1 transduction
R313-SS-1	Sm Su		-	conjugation
R313-SS-2	Sm Su		-	penicillin selection
R313-SS-3	Sm Su		(-)	P1 transduction
R313-SS-4	Sm Su		(-)	P1 transduction
R313-SS-5	Sm Su		(-)	P1 transduction
R313-SS-6	Sm Su		(-)	P1 transduction
R348-TS-1	Tc Sm	II	-	conjugation
R348-TSKC-1	Tc Sm Km Cm		+	conjugation

* ( ) indicates presumed fi character, not tested

Table 2.4.

Restrictionless mutants of the R factors of groups I & II

Mutant R factor	Parent R factor	Host specificity phenotype	Resistances	Method obtained
R124-1	R124	r^-m^+ I	Tc	EMS mutagenesis
R124-2	R124	r^-m^- I	Tc	EMS mutagenesis
R124-3	R124	r^-m^+ I	Tc	EMS mutagenesis
R132-1	R132-SS-1	r^-m^+ II	Sm Su	EMS mutagenesis
R132-2	R132-SS-1	r^-m^- II	Sm Su	EMS mutagenesis
R199-1	R199	r^-m^+ II	Tc	spontaneous
R313-1	R313	r^-m^+ II	Tc	spontaneous
R313-2	R313-T-1	r^-m^+ II	Tc	EMS mutagenesis
R313-3	R313-T-1	r^-m^+ II	Tc	EMS mutagenesis

Table 2.5.

The Bacteriophages Used in this Thesis

Phage	Requirements for adsorption	Source	Reference
λ	0.01 M Mg^{++}	S. W. Glover	Lederberg & Lederberg, 1953
λv	0.01 M Mg^{++}	S. W. Glover	Jacob & Wollman, 1954
P1	0.01 M Ca^{++}	S. W. Glover	Bertani, 1951
P2	0.01 M Ca^{++}	S. W. Glover	Bertani, 1951
ø1		S. W. Glover	Bettoni, Maccacaro & Piccinin, 1961
T1		S. W. Glover	
T5		W. Brammer	
T3		Elinor Meynell	
W31		E. Meynell	Watanabe & Okada, 1964
BF23		E. Meynell	Fredericq, 1946
MS2		E. Meynell	Davis, Strauss & Sinsheimer, 1961
If1		E. Meynell	Meynell & Lawn, 1968
ø80	0.01 M Mg^{++}	S. Brenner	Matsushiro, 1961

the R^+ strains which were not found to reduce the e.o.p. of any of the phages tested in the initial survey. Segregant R factors are listed in Table 2.3. Restrictionless mutant R factors of Groups I and II are listed in Table 2.4.

D. Bacteriophages

The bacteriophages used are listed in Table 2.5. Lysates were prepared as in chap. 2 section F, and maintained over chloroform at 4°. The term phage λ is used for phage λv. hereafter.

E. Growth and Assay of Bacterial Cultures

Overnight cultures were prepared by inoculating 5 ml. of Oxoid no. 2 broth with a single colony of the bacterial strain, followed by overnight (12 to 18 hr.) growth without aeration at 37°. Cultures prepared this way normally have a viable count of 1 to 5×10^9/ml. Log phase cultures were prepared by diluting the overnight culture 1/10 into fresh Oxoid no. 2 broth and growing them at 37° on a rotor inclined at 45°, speed 33 r.p.m. After about $1\frac{1}{2}$ hr. the cultures reached mid log phase and had titres of 2 to 5×10^8 cells/ml.

Except where otherwise stated, the cultures were assayed by serial dilution in bacterial buffer, followed by adding 0.1 ml. samples of dilutions to three ml. of water soft agar which was then poured over the surface of an Oxoid nutrient agar plate. Colonies were counted after overnight incubation at 37°.

F. Preparation and Assay of Bacteriophage Lysates

i Preparation of lysates of all bacteriophages except phage MS2

The phage lysates were prepared by the method of confluent lysis (Adams, 1950). 0.2 ml. of an overnight bacterial culture and about

10^6 phage particles were added to a tube containing three ml. of water soft agar, and the contents of the tube poured over the surface of a Difco agar plate. After overnight incubation at 37°, phage were harvested by scraping the lawn of soft agar into a sterile bottle and adding three ml. of phage buffer per lawn to extract the phage. After 20 min. extraction time at room temperature, agar and bacterial debris were removed from the lysate by two successive centrifugations. The lysate was sterilised by chloroform, and maintained over chloroform at 4°.

ii Preparation of lysates of phage MS2

A log phase culture of an F* bacterial strain was inoculated with MS2 at a multiplicity of about 0.1 and aerated until lysis occurred. The lysate was sterilised with chloroform and after bacterial debris had been removed by centrifugation, maintained over chloroform at 4°.

iii Assay of bacteriophage lysates

Phage lysates were assayed by adding 0.1 ml. of serial dilution in phage buffer to tubes containing three ml. of water soft agar and 0.2 ml. of an overnight bacterial culture. The contents of the tubes were poured over the surface of Difco agar plates which were incubated at 37°. Plaques were counted after overnight incubation.

G. Restriction Tests

Measurement of restriction was based on a comparison of the titre of a phage on a permissive strain and the test strain. Since the e.o.p. on the permissive strain is defined as 1.0, an e.o.p., regarded as a measure of the restriction, can be calculated for the test strain.

i Accurate method

Phage titrations were performed as in chap. 2 section F.iii. For each strain two plates per phage dilution were used and the e.o.p. calculated from counts on plates with between 50 and 1000 plaques.

This method gives an accurate measure of the e.o.p.

ii Quick methods

a) Lawns were prepared by adding 0.2 ml. of overnight culture to a tube containing three ml. of water soft agar and the tube contents poured over the surface of a Difco agar plate. The lawn was spotted with 0.01 ml. drops of serial ten fold dilutions of phage lysate in phage buffer and incubated overnight at 37°.

b) As in (a) above, but using hundred fold dilutions of phage lysates. This allowed the accommodation of tests for three phages on one agar plate.

c) Confluent streaks were made on a Difco agar plate using either an overnight culture, or a single colony resuspended in bacterial buffer. Three 0.01 ml. drops of hundred fold serial dilutions of phage lysate were spotted on each streak, and the plates incubated overnight at 37°. Five streaks can be accommodated per plate.

d) Confluent streaks of either a bacterial culture, or a single bacterial colony resuspended in bacterial buffer, were made on a Difco agar plate. A 0.01 ml. drop of a critical phage dilution was spotted onto the streak, and the plate was incubated overnight. Ten streaks could be accommodated per plate.

H. Modification Tests

Modification tests are based on the titration of a phage lysate, or phage from a plaque, on a known permissive strain and a known restricting strain. The e.o.p. on the permissive strain is defined as 1.0, and if the e.o.p. on the non-permissive strain is also 1.0, then the phage is said to be modified. If the e.o.p. on the restricting strain is significantly less than 1.0, then the phage is not modified.

i Accurate method

Phage titrations were performed as in chap. 2 section F.iii. This is the most accurate method of measuring e.o.p. and hence of deciding whether a phage is modified.

ii Quick method

Lawns were made by adding 0.2 ml. of overnight bacterial culture to a tube containing three ml. of water soft agar, and the contents of the tube poured over the surface of a Difco agar plate. Dilutions of the phage were spotted in 0.01 ml. amounts onto the lawns, which were then incubated overnight.

I. Measurement of Adsorption, Infective Centres, Transmission Coefficient and Burst Size

i Bacteriophage λ.K

Log phase cultures were harvested by centrifugation and prepared for λ adsorption by resuspending in λ adsorption medium at about $5x10^8$ cells/ml., and starved for 40 min. at 37°. The adsorption mixture was made by adding one ml. of phage (diluted to about 10^7 ~~bacteria~~phage/ml.) to one ml. of the starved bacterial suspension and was allowed to stand for 15 min. at 37°. Two ml. of prewarmed phage broth were added and the mixture aerated at 37° for 15 min. to facilitate phage injection. Free phage was removed from this mixture by filtration and assayed. The infected bacteria were then washed with 20 ml. of phage broth, resuspended in 10 ml. of phage broth, and the filtration, washing and resuspension procedure repeated twice. A sample of the infected bacteria was diluted and assayed for infective centres, a second sample was chloroformed and assayed for residual free phage, and a third sample was diluted a

hundred fold into pre-warmed phage broth and aerated for 90 min. before adding chloroform and assaying for phage. The burst size was then calculated.

ii Bacteriophage BF23.K

The procedure was that followed for phage λ, with the following exceptions: log phase cultures were used, without any starvation period before the 20 min. adsorption period, which was followed immediately by the filtration procedure. The sample of infected bacteria diluted into phage broth was aerated for only 40 min. before chloroform was added and the phage assayed.

J. Lysis of Bacteriophage Infected Cultures

Overnight cultures of bacterial strains were diluted 1/20 in Oxoid no. 2 broth and aerated at 37° for 1½ hr. The cultures were then adjusted to give an optical density measurement corresponding to about $2x10^8$ bacteria/ml. Ten ml. of bacterial culture and about $2x10^9$ phage particles were aerated by bubbling at 37° and the optical density of each culture was followed by taking readings at 5 min. intervals.

K. Mutagenesis with Ethyl Methane Sulphonate (EMS)

Log phase cultures were harvested by centrifugation and resuspended in one tenth the original volume of M9 buffer containing 0.02M EMS. After 60 min. the cultures were diluted 1/50 and centrifuged. The pellet was resuspended in one half the centrifugation volume of Oxoid no. 2 broth and incubated overnight. All procedures, and the overnight incubation were carried out at 30°, since the strains used were all lysogenic for the heat inducible phage λ CI_{857}. Survival after mutagenesis was between 0.1 and 1%.

L. Selection of Restrictionless Mutants

The selection procedure was performed on cultures that had been mutagenised with EMS and incubated overnight for expression. The bacterial cultures were diluted 1/10 into fresh Oxoid no. 2 broth and grown at 30° with aeration by bubbling air through the culture. Log phase cultures were diluted 1/2 into fresh Oxoid no. 2 broth containing 0.01 M Mg^{++}, Wild type phage λ was added at a multiplicity between three and five, and after a 30 min. adsorption at 30°, the culture was transferred to 42° and aerated for 60 min. Survival of heat induction was about 0.1%. The culture was diluted and plated on supplemented VB agar plates at 30°. After 48 hr. incubation, colonies were picked and tested for restriction (chap. 2 section G.ii.d). Any presumptive restrictionless colonies were further purified by two serial single colony isolations before testing for restriction, modification and for the presence of the markers of the parent strain.

M. Bacteriophage P1 Transductions

Log phase cultures in L-broth were harvested by centrifugation and resuspended in L-broth, containing 0.01 M Ca^{++}, at about 2 to 5×10^8 bacteria/ml. Phage P1 was added at a multiplicity of 0.1 and allowed to adsorb for 20 min. at 37°. The culture was centrifuged and resuspended in buffer, diluted in bacterial buffer and samples plated in three ml. of water soft agar on supplemented VB agar plates. Since the VB agar plates contain citrate, no further precaution against readsorption of the phage P1 was taken. Colonies appearing after 24 to 36 hr. incubation at 37° were picked for purification.

N. Penicillin Selection for Loss of Drug Resistance

Penicillin selection was used for selection of segregants sensitive to the bacteriostatic drugs tetracycline and sulphonamide. Selection for loss of tetracycline resistance was carried out in Oxoid no. 2 broth, but M9 minimal medium was used for selection of the loss of sulphonamide resistance. Overnight cultures were diluted to about 10^4 cells/ml. and grown with aeration by bubbling at 37° for 2 hr. then 50 µg./ml. and tetracycline, or 20 µg./ml. of sulphonamide were added. Aeration was continued for a further 60 min., and, while the culture was still less than 10^7 cells/ml., 20 µg./ml benzyl penicillin was added. After aeration at 37° for a further 4 hr., the culture was centrifuged, resuspended in bacterial buffer, diluted and plated on supplemented VB agar plates. Colonies appearing after 24 hr. growth at 37° were replica plated to supplemented VB agar containing separately each of the drugs to which the original strain had been resistant. After a further overnight incubation at 37°, colonies scored as having lost resistance to one or more drugs were picked for further purification by aerial single colony purification.

O. Transfer of Drug Resistance and the F Factor

i Measure of the frequency of drug resistance transfer

A log phase donor culture was diluted 1/10 into an overnight culture of a recipient strain. The mating mixture was returned to the inclined rotor and aerated at 37° for 30 min. The mixture was then centrifuged and resuspended in buffer, to reduce the amount of broth which is incidentally added to plates when plating the mating mixture. This is important in the case of selection for resistance to sulphonamide. Dilutions of the mating mixture were

made in bacterial buffer and 0.1 ml. samples added to tubes containing three ml. of water soft agar, which was then poured over a suitably supplemented VB agar plate. Since transfer of drug resistance was selected for, contraselection against the growth of the donor relied on omission of the requirements of the donor from the media. As controls both donor and recipient strains were plated on the selective VB agar plates. The donor culture was assayed, at the time of dilution into the mating mixture, by diluting in bacterial buffer and plating samples in 3 ml. of water soft agar on Oxoid nutrient plates. The drug resistances of the donor culture were checked by use of a multodisk (chap. 2 section P.iii) and by assaying on supplemented VB agar plates containing the drugs separately. All plates were incubated at 37° for 24 to 36 hr.

Analysis of ex-conjugants was as follows. Single colonies were picked and stabbed onto a second VB agar plate, containing the same supplements. This template was incubated overnight at 37°, and then replica plated to supplemented VB agar plates containing separately the drugs to which the donor strain had been resistant. The replica plates were then incubated overnight at 37°. The colonies to be tested for restriction of phage λ were picked from a replica plate (if possible) and streaked to single colonies on supplemented VB agar plates before picking a single colony for the test. The restriction test used was that detailed in chap. 2 section G.ii.d.

ii Preparation of R^+ strains

When an R^+ strain was made, but no knowledge of the frequency of transfer was required, the following procedure was used. Equal

volumes of a log phase donor culture and an overnight recipient culture were mixed and placed on a rotor at 37° for 60 min. The mating mixture was then streaked onto selective VB agar plates and incubated for 24 to 36 hr. Single colonies were carried through two single colony isolations on selective VB agar plates. A single colony was then picked to test for drug resistance and the characteristics of the recipient strain. Generally four or five colonies were taken through the procedure to this stage, one of which was selected as the stock strain.

iii Transfer of F⁺ or F' factors

The procedure for transfer of an R factor was followed. The presence of F in the colony finally selected was tested by both the ability of a culture to plate MS2, and by a reduction in the e.o.p. of phage T3. For strains which carried an fi⁺ R factor, only the test with phage T3 was carried out.

P. Tests for Drug Resistance

i Multodisk method

0.2 ml. of an overnight culture to be tested was added to a tube containing three ml. of water soft agar, and the contents of the tube poured over an agar plate (nutrient agar was used unless resistance to sulphonamide was to be tested, in which case the medium used was supplemented VB agar). A multodisk was placed on the water soft agar lawn, and the plate incubated overnight. A clear halo round the part of the multodisk containing a drug indicated sensitivity to the drug, whereas growth of the bacterial lawn up to the edge of the paper indicated resistance to the drug. This method had the advantage of allowing the resistance of a culture to all the drugs used in this thesis to be tested on the same plate.

ii Streak method

A loopful of culture, or of a colony resuspended in bacterial buffer, was streaked on supplemented VB agar plates containing separately each of the drugs to which resistance was to be tested, care being taken to streak to single colonies whenever possible. Growth after 24 to 36 hr. incubation indicated resistance to the drug. This method was reliable for testing for sulphonamide resistance. Up to ten colonies or cultures could be streaked on one plate.

iii Replica plating method

The colonies under test, either as stabs on a template or as single colonies on a spread agar plate, were replica plated to supplemented VB agar plates containing separately the appropriate drugs. The replica plates were incubated overnight at 37° and growth on a replica plate was taken to indicate resistance to the drug in the plate. This method cannot be used to test for sulphonamide resistance. As many as a hundred colonies or 50 stabbed colonies on a template can be tested on one plate.

Q. Detection of Colicin I Production and Resistance to Colicin Ib

i Detection of colicin I production

Single colonies of the strain under test were stabbed onto duplicate Oxoid nutrient agar in glass petri dishes and incubated overnight at 37°. The bacteria were killed by placing a few drops of chloroform on the lid of the glass petri dish, and leaving the plate closed for 30 min. at room temperature. After evaporation of the chloroform from the lid, the agar was overlayed with three ml. of water soft agar containing 0.2 ml. of overnight culture. One

of each pair of petri dishes was overlayed using a colicin I resistant strain. The plates were incubated overnight at 37°. Only those stabs surrounded by a clear zone of inhibition of the sensitive indicator bacteria, but showing no inhibition of the resistant bacteria, were recorded as colI*. On each plate a none colicinogenic colony and a colI+ colony were included as controls.

ii Resistance to colicin Ib

Colonies of a colIb* colicin I resistant strain were stabbed onto Oxoid nutrient agar in small glass petri dishes. After overnight incubation at 37°, the bacteria were killed by placing a few drops of chloroform on the lid of the petri dish, and leaving the petri dish closed for 30 min. at room temperature. After evaporation of the chloroform, each plate was overlayed with 1.5 ml. of water soft agar containing 0.1 ml. of a test culture. The test series always included a colicin I sensitive culture and a colicin I resistant culture as controls. After overnight incubation at 37°, those cultures which had grown over the stab of the colIb* strain were said to be colicin Ib resistant, and those which had a clear zone of inhibition around the stab were said to be colicin Ib sensitive.

UNIV. EDIN. GEN EX BIBL

CHAPTER 3

SURVEY, GROUPING AND PRELIMINARY INVESTIGATION OF R^+ STRAINS

A. Introduction

i Bacteria

All work in this thesis was carried out using strains of *Escherichia coli*. The e.o.p. of a phage is exceedingly strain, even sub-strain, dependent and to avoid scoring trivial differences in plating efficiency, the R^+ strains used in the survey were derivatives of a single bacterial strain, J5-3 *pro*$^-$*met*$^-$(λ)F^-. These J5-3 R^+ strains were the gift of Drs. Naomi Datta and Elinor Meynell, and had been prepared by transfer of drug resistance from resistant isolates of *Salmonella* and *E. coli*.

ii Bacteriophages

Even though the techniques used to survey the R^+ strains were both rapid and accurate (chap. 2 section G.ii.b), the maximum number of phages which could be examined on one plate was three; with 153 R^+ strains to screen, it was decided to limit the number of phages used to eight. Of these four (phages λ, ϕ80, P1 and P2) were known to be subject to at least one host specificity; two (phages λ and P1) had been reported to be restricted by R factors (see chap. 1 section II.E). Phage BF23, apart from sharing the receptor site of the E colicins, was known to have a reduced e.o.p. on *colIb*$^+$ strains and on R^+ strains which were resistant to colicin Ib (chap. 1 section II.E). The other three phages chosen for the original survey (phages T3, W31 and ϕ1) all belong to the class of phages termed "female specific" because the e.o.p. is reduced on F^+ strains. Phage W31 had already been reported to have

a reduced e.o.p. on some R^+ strains (chap. 1 section II.E). Although all three phages were used in the survey, the plating efficiencies for each phage on the same strain were so close that they are represented in the text by one phage only, phage ø1.

Two other phages have been used, phages T1 and T5. Phage T1 is subjected to the host specificity carried by phage P1, but not by the *E. coli* chromosomal K and B types (Eskridge *et al.*, 1967), and therefore was tested on groups I and II. Nisioka and Ozeki (1968) reported that phage T5, as well as phage BF23, had a reduced e.o.p. on *colIb*$^+$ strains, hence this phage was tested on those strains which reduce the e.o.p. of BF23.

iii The techniques

An R^+ strain which both restricted and modified a phage was easily recognised and the host specificity characterised. In cases where reductions in e.o.p. were not accompanied by modification, it was necessary to use other methods to decide whether the strain should be termed restricting. The techniques used for this purpose are listed below; the phage techniques were only used with phages λ, T3 and BF23, since the reductions in e.o.p. of phages ø80, P1 and P2 were accompanied by a reduction in e.o.p. of phage λ (exception group VII).

a) Screening the R^+ strains

The initial survey of the R factors used the method in chap. 2 section G.ii.b. Accurate e.o.p. measurements on each of the eight phages was made by the method of chap. 2 section G.i. on all the R^+ strains lowering the e.o.p. of any phage in the initial survey. This incidentally provided a control for the efficiency of the initial survey.

b) Modification

Phage from plaques on R^+ strains showing a reduced e.o.p. of the phage were tested for modification by the method of chap. 2 section H.ii; the phage were also checked not to be mutants. If modification was found it was accurately measured by the method in chap. 2 section H.i.

c) Measurement of adsorption, infective centres, transmission coefficient and burst size

The method used is detailed in chap. 2 section I. Typically, the only feature of phage infection and growth which is altered in a restricting host is the production of infective centres, and hence transmission coefficient. Two other features of phage infection which can account for reduced e.o.p. are poor adsorption and low burst size. Adsorption of the female specific phages was too poor to allow this method to be used, and no antisera were available.

d) Lysis of bacteriophage infected cultures

Premature lysis, delayed lysis, or failure of lysis of the phage infected cell could be responsible for a reduction in e.o.p. Premature lysis has been found for phage BF23 on colIb$^+$ strains (Nisioka and Ozeki, 1968) and a similar method (chap. 2 section J) was used here for both phages BF23 and T3.

e) Isolation of bacteriophage mutants

When the e.o.p. of a phage on an R^+ strain was very low there was selection for phage mutants able to grow on the R^+ strain. Such spontaneous mutants were of use in confirming the designation of an R^+ strain to a particular group.

f) Transfer of the genetic determinants for reduction in e.o.p.

A major interest in the isolation of new host specificities associated with R factors was the potential ease of transfer of

the genetic determinants in association with the drug resistances. Transfer was demonstrated by using the method of chap. 2 section O.ii, and screening the drug resistant ex-conjugants for reduction in e.o.p. Where this failed, a mating mixture was diluted and grown overnight at 37° before selection and testing, but no strain failing to transfer reduction in e.o.p. by the normal method did so under these conditions.

B. The R^+ Strain Groups - Experimental Results

The results of the initial survey indicated that 61 of the 153 R^+ strains tested reduced the e.o.p. of one or more phages. Although it was not intended to imply a close relationship between the R^+ strains grouped together on the basis of patterns and levels of e.o.p., the 61 R^+ strains did fall clearly into ten groups, apparently homogeneous (with the possible exception of group X). Table 3.1. summarises the groups, e.o.p. being expressed as less than (<) where abnormalities of plaque morphology do not permit an accurate measure to be made. The results will be dealt with according to the R^+ strain groups, but for reasons which will become apparent the order of the groups will not be that in Table 3.1. The numbering system of Table 3.1. is retained to conform with published work (see appendix).

i Group I

Only one R^+ strain, J5-3 R124, was assigned to group I on the basis of the characteristic restrictions and modification expressed in Tables 3.2. to 3.7. Of the original survey phages, four (phages λ, ø80, P1 and P2) were restricted and modified, and the phage T1 was also restricted and modified. Any of these five phages were restricted on J5-3 R124, unless the last host was J5-3

Table 3.1.

The Efficiencies of Plating of Bacteriophages on the J5-3 R^+ Groups

Group	Bacteriophages						Number of R^+ strains		
	ø1	BF23	λ	ø80	P2	P1	fi^-	fi^+	not tested
I	1.0	1.0	4×10^{-4}	1×10^{-4}	3×10^{-5}	1×10^{-1}		1	
II	1.0	1.0	2×10^{-2}	7×10^{-3}	6×10^{-1}	7×10^{-4}	9	2	
III	1.0	1.0	$<10^{-2}$	$<10^{-1}$	1.0	1.0		4	
IV	1.0	1.0	$<10^{-2}$	1.0	1.0	1.0		1	
V	$<10^{-3}$	3×10^{-1}	$<10^{-2}$	1.0	1.0	1.0	3		
VI	$<10^{-3}$	$<10^{-3}$	$<10^{-2}$	1.0	1.0	1.0	1		
VII	1×10^{-1}	5×10^{-1}	1.0	1.0	1.0	7×10^{-7}		2	
VIII	$<10^{-3}$	1×10^{-1}	1.0	1.0	1.0	1.0	3	1	
IX	6×10^{-1}	$<10^{-3}$	1.0	1.0	1.0	1.0	8	3	
X	1×10^{-1}	5×10^{-1}	1.0	1.0	1.0	1.0	18	3	2
others	1.0	1.0	1.0	1.0	1.0	1.0	32	51	9

Table 3.2.

Efficiencies of plating of J5-3 grown phages on strains J5-3 R⁻ and J5-3 R124

Strain	Phage λ	ø80	P1	P2	T1
J5-3 R⁻	1.0	1.0	1.0	1.0	1.0
J5-3 R124	4×10^{-4}	1×10^{-4}	1×10^{-1}	3×10^{-5}	1×10^{-3}

Table 3.3.

Efficiencies of plating of bacteriophage λ grown on J5-3 R⁺ strains

Group of host	Group of the last host strain of the bacteriophage: -	I	II	III	IV, V or VI
-	1.0	1.0	1.0	1.0	1.0
I	4×10^{-4}	1.0	2×10^{-4}	1×10^{-5}	2×10^{-4}
II	2×10^{-2}	1×10^{-2}	1.0	2×10^{-2}	2×10^{-2}
III	$< 10^{-2}$	$< 10^{-2}$	$< 10^{-2}$	$< 10^{-2}$	$< 10^{-2}$
IV, V or VI	$< 10^{-2}$	$< 10^{-2}$	$< 10^{-2}$	$< 10^{-2}$	$< 10^{-2}$

Table 3.4.

Efficiencies of plating of bacteriophage ø80 grown on J5-3 R⁺ strains

Group of host	Group of the last host strain of the bacteriophage: -	I	II	III
-	1.0	1.0	1.0	1.0
I	1×10^{-4}	1.0	1×10^{-4}	1×10^{-4}
II	7×10^{-3}	4×10^{-3}	1.0	3×10^{-3}
III	$< 10^{-2}$	$< 10^{-2}$	$< 10^{-2}$	$< 10^{-2}$

Table 3.5.

Efficiencies of plating of J5-3 R+ grown bacteriophage P2

Group of host	Group of the last host strain of the bacteriophage		
	-	I	II
-	1.0	1.0	1.0
I	3×10^{-5}	1.0	6×10^{-4}
II	6×10^{-1}	5×10^{-1}	1.0

Table 3.6.

Efficiencies of plating of J5-3 R+ grown bacteriophage P1

Group of host	Group of the last host strain of the bacteriophage			
	-	I	II	VII
-	1.0	1.0	1.0	1.0
I	1×10^{-1}	1.0	3×10^{-1}	4×10^{-1}
II	7×10^{-4}	3×10^{-4}	1.0	1×10^{-3}
VII	7×10^{-7}*	$< 10^{-5}$	$< 10^{-5}$	1.0*

* These phages are mutants

Table 3.7.

Efficiencies of plating of J5-3 R+ grown bacteriophage T1

Group of host	Group of the last host strain of the bacteriophage		
	-	I	II
-	1.0	1.0	1.0
I	1×10^{-3}	1.0	1×10^{-3}
II	7×10^{-4}	4×10^{-4}	1.0

Table 3.8.

Infection of R^+ Strains with phage λ.J5-3:

Results of two experiments

Strain	Group	% Adsorption	Infective Centres/ml.	% Transmission	Burst size
J5-3 R^-	-	78	$3x10^7$	71	58
		76	$3x10^7$	74	54
J5-3 R124	I	83	$6x10^3$	0.01	34
		90	$2x10^4$	0.04	32
J5-3 R203	II	90	$9x10^5$	2	26
		99	$7x10^5$	1	66
J5-3 R230	III	82	$3x10^6$	5	36
		85	$2x10^6$	6	26
J5-3 R276	IV	89	$2x10^6$	3	3
		91	$2x10^6$	3	2
J5-3 R64	V	79	$3x10^6$	7	0.4
		99	$3x10^6$	7	0.1
J5-3 R252	VI	94	$1x10^6$	3	0.6
		97	$5x10^6$	10	0.3

Input bacteriophage in the first experiment were $6x10^7$/ml., and in the second experiment $5x10^7$/ml.

R124 (second column, tables 3.3 to 3.7); and phages grown on J5-3 R124 still had a reduced e.o.p. on other hosts which reduce the e.o.p. of J5-3 R⁻ grown phages (see the second lines of tables 3.3. to 3.7.). Hence it was concluded that the specificity of restriction and modification imposed by this strain was different to that imposed by the group II strains, and that there was no detectable relationship with any other strain causing a change in e.o.p. of any of the five phages.

Adsorption of phage λ.J5-3 to J5-3 R124 was normal, as was the burst size, but infective centre formation and transmission coefficient were greatly reduced (Table 3.8). The reduction in infective centre formation corresponded with the reduction in e.o.p., which is typical of cases where the reduction in e.o.p. is due to host controlled modification.

On the basis of this data, it was concluded that the strain J5-3 R124 carried determinants for a host specificity, and that this host specificity was distinct from that determined by the group II strains. The genetic determinants for this host specificity are from here on represented by the symbol hsI. Transfer of hsI in association with tetracycline resistance, the only drug resistance carried by this R factor, has been demonstrated (see chap. 4 section I.C), so it has been assumed that hsI is carried on the R factor, which will also be referred to as RI. The J5-3 R124 strain has been checked to be fi⁺, colicin sensitive and to produce no colicin.

Table 3.9.

Plating efficiencies of restricted, J5-3 R⁻ grown, phages on group II strains

Strain	Phage λ	ø80	P1	P2	T1
J5-3 R⁻	1.0	1.0	1.0	1.0	1.0
J5-3 R132	1×10^{-2}	6×10^{-3}	$< 10^{-8}$	$< 10^{-6}$	8×10^{-4}
J5-3 R199	2×10^{-2}	8×10^{-3}	3×10^{-4}	4×10^{-1}	1×10^{-3}
J5-3 R203	2×10^{-2}	8×10^{-3}	3×10^{-4}	3×10^{-1}	1×10^{-3}
J5-3 R204	2×10^{-2}	6×10^{-3}	5×10^{-4}	3×10^{-1}	1×10^{-4}
J5-3 R245	2×10^{-2}	8×10^{-3}	1×10^{-3}	4×10^{-1}	1×10^{-4}
J5-3 R250	2×10^{-2}	1×10^{-2}	4×10^{-4}	6×10^{-1}	1×10^{-3}
J5-3 R265	2×10^{-2}	6×10^{-3}	2×10^{-3}	4×10^{-1}	3×10^{-4}
J5-3 R269	2×10^{-2}	8×10^{-3}	2×10^{-3}	3×10^{-1}	1×10^{-3}
J5-3 R270	2×10^{-2}	7×10^{-3}	6×10^{-4}	4×10^{-1}	1×10^{-3}
J5-3 R313	3×10^{-2}	5×10^{-3}	3×10^{-4}	3×10^{-1}	3×10^{-4}
J5-3 R348	2×10^{-2}	8×10^{-3}	7×10^{-4}	4×10^{-1}	1×10^{-3}

ii Group II

The strains assigned to group II showed a characteristic reduction in e.o.p. of four of the phages used in the survey (phages λ, ø80, P1 and P2), and for phage T1 (see table 3.9.). The group II strains also modify the five phages, and the host specificity was found to be identical for all 11 strains, and to extend to all five phages. A single illustration is given in Table 3.10., where it is clear that although phage λ.J5-3 was restricted on all the group II strains, phage λ.J5-3 R203 was modified so that it was not restricted by any group II strain.

Table 3.10.

Modification of phage λ by a group II strain

Host strain	Efficiency of plating of phage	
	λ.J5-3	λ.J5-3 R203
J5-3 R⁻	1.0	1.0
J5-3 R132	1×10^{-2}	1.0
J5-3 R199	2×10^{-2}	0.9
J5-3 R203	2×10^{-2}	1.0
J5-3 R204	2×10^{-2}	1.0
J5-3 R245	2×10^{-2}	0.9
J5-3 R250	2×10^{-2}	0.9
J5-3 R265	2×10^{-2}	1.0
J5-3 R269	2×10^{-2}	1.0
J5-3 R270	2×10^{-2}	1.0
J5-3 R313	3×10^{-2}	1.0
J5-3 R348	2×10^{-2}	1.0

Phage grown on group II strains plated with an e.o.p. of 1.0 on strains of group II, or none restricting strains (second column, tables 3.3. to 3.7.). Likewise only group II grown phages were not restricted by group II strains (line three, tables 3.3. to 3.7.). Hence the host specificity associated with the group II strains was not that of group I, nor was it in any way related to any other reduction in e.o.p. of these phages found on other strains.

When tested with phage λ.J5-3, adsorption and burst size were normal for the group II strain J5-3 R203, but the production of infective centres and transmission coefficient were reduced (table 3.8.). It was therefore concluded that group II strains are associated with a host specificity whose genetic determinants will be referred to as hsII, and the general symbol RII will be used for any R factor carrying hsII. Transfer of this host specificity hsII to recipients by conjugation can occur (see chap. 4). All except two of the strains proved to be fi^- on testing, and the fi^+ character of the other two strains, J5-3 R269 and J5-3 R348, will be dealt with in chapter 4, as will be the apparent resistance of J5-3 R132 to phages P1 and P2.

iii Group III

The four strains assigned to group III reduced the e.o.p. of phages λ and ø80, but the plaques were so tiny and indistinct that the e.o.p. could only be expressed as less than (<) a figure believed to represent an upper limit for the e.o.p. (table 3.11.). No modification of either phage was observed, and both phage λ grown on groups I to VI, and phage ø80 grown on groups I and II, still have a low e.o.p. on strains of group III (fourthcolumn, tables 3.3. and 3.4.). Nor did group III strains improve the e.o.p. of either phage on groups I to VI (fourth line tables 3.3. and 3.4.), so there

Table 3.11.

Efficiencies of plating of J5-3 R⁻ grown bacteriophages on group III strains

Host strain	Efficiency of plating of phage		
	λ	ø80	λIII*
J5-3 R⁻	1.0	1.0	1.0
J5-3 R230	$<10^{-2}$	$<10^{-1}$	1.0
J5-3 R231	$<10^{-2}$	$<10^{-1}$	1.0
J5-3 R234	$<10^{-2}$	$<10^{-1}$	1.0
J5-3 R329	$<10^{-2}$	$<10^{-1}$	1.0

* λIII is a mutant phage λ able to grow on group III strains

Table 3.12.

Efficiency of plating of bacteriophage λIII* on groups III to VI

Group of host strain				
-	III	IV	V	VI
1.0	1.0	$<10^{-2}$	$<10^{-2}$	$<10^{-2}$

is no apparent connection between group III and any other group.

Although no modification was observed, mutants of phage λ, designated λIII, were easily obtained at a frequency of about 10^{-6} per phage λ. The six independently isolated mutants behaved identically, all plating on all group III strains, irrespective of the strain isolated on, and having no improved e.o.p. on any of the other three strain groups that do not modify phage λ, i.e. groups IV, V and VI

(Tables 3.11. and 3.12.).

Adsorption of phage λ.J5-3 was normal to group III strains (table 3.8.), but transmission coefficient was reduced as might have been expected if this was a restricting but non-modifying strain. However, these strains, though obviously a homogeneous group on the basis of the plating of the mutant phages λIII, were not further investigated when it did not prove possible to transfer the reduction in e.o.p. out of the strains in connection with the drug resistance. All four strains were checked to be fi$^+$.

iv Group IV

A single R$^+$ strain, J5-3 R276, checked to be fi$^+$, was found which reduced the e.o.p. of phage λ. Tiny, indistinct plaques prevented an accurate measure of the e.o.p., but it was less than 10^{-2}. Phage λ grown on any other of the groups I to VI was not improved in e.o.p. on this strain (fifth column, table 3.3.), nor did phage λ.J5-3 R276 have a normal e.o.p. on strains of groups I to VI (fifth line, table 3.3.). Adsorption of λ.J5-3 to this strain was normal, but the transmission coefficient was reduced and the burst size measured was so low as to suggest that there was no burst under the experimental conditions (table 3.8.). Hence J5-3 R276 is a non-modifying strain, but the reduction in e.o.p. is likely to be due to some disruption of the normal process of phage development in the infected cell. The strain was not further investigated when it did not prove possible to transfer the reduction of phage λ e.o.p. out of the strain in conjunction with any drug resistance, although a segregant colony was found which did not reduce the e.o.p. of phage λ.

v Groups VI and IX

The common characteristic of groups VI and IX was a much reduced e.o.p. of phage BF23, with plaques so tiny and indistinct as to

prevent an accurate measure of the e.o.p. (tables 3.13. and 3.14.). Group VI was only represented by one R^+ strain, checked to be fi^-, J5-3 R252. As seen in table 3.13, this strain also reduced the e.o.p. of phage Ø1 drastically, and also reduced the e.o.p. of phage λ. The reduced e.o.p. of phage λ resembled that seen with the group IV strain, and was never transferred out of the strain, so will not be discussed further here (refer particularly to tables 3.3. and 3.8. for details). Group IX strains differed from the group VI strain in only reducing the e.o.p. of Ø1 slightly, though plaques are abnormal (table 3.14.). Although ex-conjugants, when J5-3 R252 was used as donor, always reduced the e.o.p. of Ø1 drastically, and no separation into two plasmids was achieved, group VI will still be considered with group IX. In investigation of lysis of T3 infected bacteria, the group IX strain was indistinguishable from the control, but the very low e.o.p. of the female specific phages on the group VI strain was apparently related to a failure of the infected cells to lyse (Fig. II).

The reduced e.o.p. of both phages BF23 and Ø1 could be transferred out of the strains in both groups VI and IX. Adsorption of BF23.J5-3 to representative strains of groups VI and IX was normal, infective centre formation and transmission coefficient were reduced about 10 fold, but burst size was probably reduced to zero (table 3.15.). In experiments in which lysis of phage infected cultures was followed turbidimetrically, both strains of group VI and group IX infected with phage BF23 lysed early, at 15 min. Control J5-3 infected cultures lyse at about 40 min., while <u>colIb</u>$^+$ cultures also lyse at 15 min. (Fig. 1). This early lysis of <u>colIb</u>$^+$ cultures was found by Nisioka & Ozeki (1968), and ten of the 11 group IX strains proved to be <u>colIb</u>$^+$.

Table 3.13.

Characteristics of Group VI

Strain	Efficiency of plating of bacteriophage			colicin Ib* production	Resistance** to colicin Ib
	ø1.J5-3	BF23.J5-3	λ.J5-3		
J5-3 R252	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-2}$	-	R

* - indicates no colicin Ib production
** R indicates resistance to colicin Ib

Table 3.14.

Characteristics of Group IX

Strain	Efficiency of plating of bacteriophage		colicin Ib* production	Resistance** to colicin Ib
	ø1.J5-3	BF23.J5-3		
J5-3 R39	0.9	$< 10^{-3}$	+	R
J5-3 R62	0.4	$< 10^{-3}$	+	R
J5-3 R143	0.1	$< 10^{-3}$	+	R
J5-3 R144	0.2	$< 10^{-3}$	+	R
J5-3 R145	0.9	$< 10^{-3}$	+	R
J5-3 R163	0.6	$< 10^{-3}$	+	R
J5-3 R193	0.9	$< 10^{-3}$	+	R
J5-3 R194	0.9	$< 10^{-3}$	+	R
J5-3 R241	0.6	$< 10^{-3}$	+	R
J5-3 R324	0.8	$< 10^{-3}$	+	R
J5-3 R330	0.7	$< 10^{-3}$	-	S

* + indicates production of colicin Ib, - failure to produce colicin Ib
** R indicates resistance, S sensitivity to colicin Ib

Table 3.15.

Infection with BF23.J5-3:

Results of two experiments

Strain	Group	% Adsorption	Infective Centres/ml.	% Transmission	Burst size
J5-3 R$^-$	-	94	$4x10^6$	18	88
		96	$4x10^6$	8	381
J5-3 R64	V	99	$7x10^6$	37	33
		96	$4x10^6$	7	137
J5-3 R252	VI	89	$2x10^5$	1	<2
		89	$3x10^5$	0.6	3
J5-3 R191	VII	96	$8x10^6$	41	88
		72	$2x10^6$	6	245
J5-3 R309	VIII	95	$4x10^6$	20	33
		97	$4x10^6$	8	385
J5-3 R163	IX	100	$3x10^4$	0.1	5
		98	$7x10^4$	0.1	NS

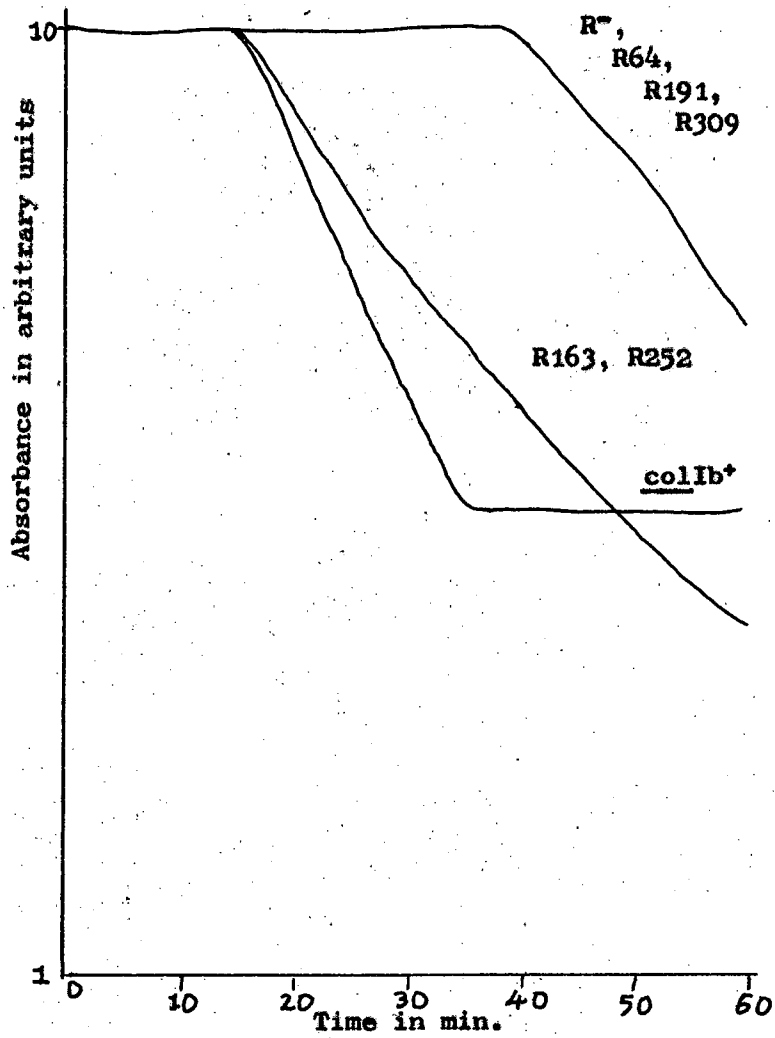
Input phage in the first experiment was $2x10^7$/ml., in the second experiment $5x10^7$/ml.

NS indicates not scorable because of the high contamination of the infective centres with residual free phage.

Fig. I

Lysis of phage BF23-infected

J5-3 R⁺ and J5-3 colIb⁺ cultures

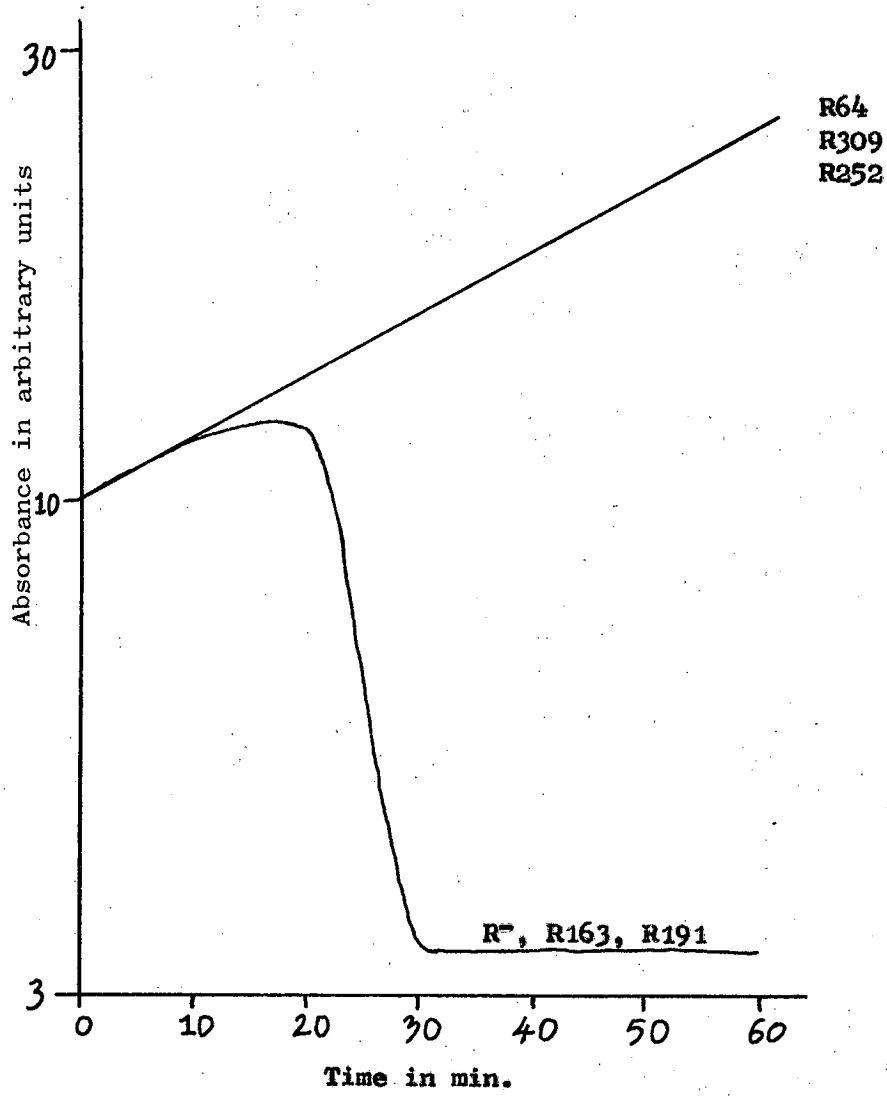


For method see chap. 2 section J

Fig. II

Lysis of phage T3 infected

J5-3 R^+ cultures



For method see chap. 2 section J

Table 3.16A

Efficiencies of plating of J5-3 grown bacteriophages BF23 and BF23*

Host strain	Phage BF23	Phage BF23*
J5-3 R⁻	1.0	1.0
J5-3 R252	$< 10^{-3}$	1.0
J5-3 RGIX**	$< 10^{-3}$	1.0
J5-3 colIb*	$< 10^{-3}$	1.0

**J5-3 RGIX indicates any group IX strain

Table 3.17.

Characteristics of group V strains

Strain	Efficiency of plating of J5-3 grown phage			colicin Ib* production	colicin Ib* resistance
	ø1	BF23	λ		
J5-3 R41	$< 10^{-3}$	0.3	$< 10^{-2}$	-	S
J5-3 R56	$< 10^{-3}$	0.4	$< 10^{-2}$	-	S
J5-3 R64	$< 10^{-3}$	0.2	$< 10^{-2}$	-	S

*- indicates no production, S indicates sensitivity

Table 3.18

Characteristics of group VIII strains

Strain	Efficiency of plating of phage		colicin Ib* production	colicin Ib* resistance
	ø1.J5-3	BF23.J5-3		
J5-3 R309	$< 10^{-3}$	0.2	-	S
J5-3 R318	$< 10^{-3}$	0.2	-	S
J5-3 R340	$< 10^{-3}$	0.1	-	S
J5-3 R341	$< 10^{-3}$	0.1	-	S

* - indicates no colicin Ib production, S indicates sensitivity to colicin Ib

The remaining strain is neither colIb* nor colicin Ib resistant, while the group VI strain is not colIb*, but is colicin Ib resistant (tables 3.13. and 3.14.). While the correlation is not absolute it is suggested that the phenomenon observed here is due to colIb in the bacteria, and as no analysis of the strains have been performed, it is not possible to say that the colIb is or is not associated with the R factor (see chap. 3 section C).

This evidence is supported by the existence of mutants of phage BF23, termed phage BF23*, able to plate normally on groups VI and IX strains. A total of 14 independent isolates were tested, and whether selected on group VI or on a group IX strain, all plate with equal efficiency on J5-3, the group VI strain, all group IX strains and the colIb* strain (Table 3.16.).

vi Groups V and VIII

The strains of both groups V and VIII showed a greatly reduced e.o.p. of phage ∅1, no accurate measure of the e.o.p. being available, and a slight reduction in e.o.p. of phage BF23, accompanied by a slight decrease in plaque diameter. The difference between the two groups was a reduced e.o.p. of phage λ by group V, resembling that found with group IV, and likewise not transferred from the R* strains on selection for drug resistant ex-conjugants. It will therefore not be discussed further here (see tables 3.3. and 3.8.). The group V strains did transfer the effects on the e.o.p. of phage ∅1 and phage BF23 when drug resistance is transferred, as do the strains of group VIII. These two groups are therefore considered together. There was no evidence of abnormality in adsorption or transmission coefficient for phage BF23 in these strains, though the burst size was slightly lower than control values ;33 and 37 against the

control 88 in one experiment, for example (table 3.15.). As the e.o.p. of phage BF23 was only slightly lowered, this reduction in burst size may account for the effect on the e.o.p. No abnormality was observed for either group in experiments on lysis of phage BF23 infected cultures (Fig. I). All strains were colicin Ib sensitive and did not produce colicin Ib, so there is no relation of the reduction in e.o.p. of phage BF23 with colIb as with groups VI and IX.

With regard to the reduction in e.o.p. of the female specific phages, the only information is that phage T3 infected cultures did not lyse at the normal control time, and in fact appeared to grow normally (Fig. II).

vii Groups VII and X

The common features of these two groups were the slight reductions in e.o.p. of both phages ø1 and BF23, accompanied by plaque abnormalities, more marked in the case of ø1 (Tables 3.19. and 3.20.). Group VII also reduced the e.o.p. of phage P1, the only phage growing on the two strains being mutants (see table 3.6.) However, this effect has not been transferred out of the strains with the drug resistances, and will not be discussed further. It is of interest, however, that in these transfer experiments two classes of ex-conjugant were found, both carrying resistance to all the drugs of the donor, but only one showing the reduction in e.o.p. of phages ø1 and BF23 characteristic of group X strains.

As might be expected from the low level of the effect on the e.o.p. of phages ø1 and BF23, no abnormality was detected in lysis of phage infected cultures (Fig. I and II), or in adsorption, transmission coefficient or burst size for phage BF23 (table 3.15.).

Table 3.19.

Efficiencies of plating of J5-3 grown bacteriophages on Group VII

Host strain	Bacteriophage Ø1	BF23	P1
J5-3 R⁻	1.0	1.0	1.0
J5-3 R191	0.7	0.6	5×10^{-7}*
J5-3 R196	0.7	0.4	5×10^{-7}*

* These phages are mutants

Table 3.20.

Efficiencies of plating of J5-3 grown bacteriophages on Group X

Strain	Bacteriophage Ø1	BF23	Strain	Bacteriophage Ø1	BF23
J5-3 R⁻	1.0	↑	J5-3 R301	0.1	↑
J5-3 R70	0.2		J5-3 R302	0.09	
J5-3 R73	0.1	about	J5-3 R303	0.1	
J5-3 R75	0.07	0.5	J5-3 R304	0.1	
J5-3 R77	0.09		J5-3 R305	0.1	about
J5-3 R243	0.07		J5-3 R306	0.2	0.5
J5-3 R295	0.1		J5-3 R307	0.1	
J5-3 R296	0.1		J5-3 R310	0.1	
J5-3 R297	0.1		J5-3 R311	0.1	
J5-3 R298	0.1		J5-3 R336	0.08	
J5-3 R299	0.05		J5-3 R337	0.1	
J5-3 R300	0.03	↓	J5-3 R338	0.07	↓

These reductions in e.o.p. were transmissible with drug resistance from the original strains, but no explanation is available for them.

C. Discussion

i The R+ strain grouping

It is appropriate here to re-emphasise a few points before further discussion. While the intention of the survey was not to find characters for the classification of R factors, the 61 R factors affecting the e.o.p. of one or more phages could be divided into ten groups based on the pattern of bacteriophages affected, supplemented by the precise e.o.p. observed. For example, the strains of groups VIII and IX reduced the e.o.p. of both phage BF23 and phage ∅1, but the two groups were separated because four R+ strains (group VIII) drastically reduced the e.o.p. of phage ∅1 while lowering the e.o.p. of phage BF23 only slightly, the converse being true of the strains designated to group IX. Although both e.o.p. and plaque morphology can vary widely with plating conditions, the distinction between a slightly reduced and a greatly reduced e.o.p. is very clear for any given phage.

It was convenient to continue the examination of the R+ strains in the ten groups which are groups of J5-3 R+ strains. This grouping does not necessarily imply any relationship between the R factors carried by these R+ strains. In some cases the genetic determinant producing a particular effect on a phage e.o.p. has been shown to be associated with several R factors (e.g. the hsII determinant), but a close relationship between R factors carried by R+ strains of a particular group cannot be assumed in all cases. It has been suggested (Anderson, 1966) that genetic determinants other than the resistance determinants might be used for classifying or subdividing

R factors. Alternatively, the genetic determinants associated with an R factor might be indicative of the "past history" of the R factor. For example, Anderson and Lewis (1965) found an R factor, whose presence in a Salmonella strain caused the same phage-type change as lysogenisation for a Salmonella phage. A drug sensitive derivative of the R factor retained both sex factor activity and phage-type determinants. This particular case may be argued as evidence for an evolutionary relationship between the R factor and the phage. It also illustrates the potential use of R-factor associated determinants as markers for the investigation of sex factors after dissociation from the resistance determinants. The discovery, recorded in this thesis, that non-transmissible determinants may at first sight appear to be associated with R factors emphasises the need for investigation of direct derivatives of drug resistant isolates before using any apparent determinant for a classification of R factors. It is possible that the use of other phages, or use of other Enterobacteriaceae, as host bacteria, would have provided evidence for further subdivision of the groups presented here. However, with the possible exception of group X, the groups appear to be homogeneous with respect to several characters.

Watanabe et al. (1966) stated that only fi⁻ R factors are associated with reductions in e.o.p. of phages. The total number of R factors surveyed here is much greater than in any other investigation previously reported using Escherichia coli strains; hence this survey could be expected to reveal any such association. Of the 153 R⁺ strains surveyed (table 3.1.) 76 were fi⁻ and 66 were fi⁺, and all were not scored for fi character. Of the 61 R⁺ strains which affect the e.o.p. of one or more phages, 44 are known to be fi⁻, and the proportion of known fi⁻ R factors is even higher if only

transmissible effects are considered, 44 out of 58 R^+ strains being known to be *fi*$^-$. Out of the remaining 14 R^+ strains, the *fi* character of two is not known, and a minimum of seven harbour an *fi*$^-$ as well as an *fi*$^+$ R factor. An additional interesting finding is that an *fi*$^+$ R factor carried by J5-3 R62 (group IX) determines an I-type pilus (Romero & Meynell, 1969). It seems that this connection between *fi*$^-$ character and the carrying of determinants reducing the e.o.p. of phages may well be general; but it could be the production of the I-type pilus, rather than *fi* character as such (which is a measure of the sensitivity of F to be repressed by the R factor) which is involved.

A notable exception to this rule is the R factor R124. This R factor has been shown to be *fi*$^+$ and to determine the F-type pilus (Lawn *et al.*, 1967), and the strain J5-3 R124 was checked here to be *fi*$^+$. This R factor is also unique in this collection in being the only one associated with the hsI determinant. It is a fact that the F factor itself reduces the e.o.p. of the female specific phages (Dettori *et al.*, 1961).

ii The non-transferable e.o.p. reductions

Two groups (groups III and IV) have not been shown to transfer effects on e.o.p. by conjugation. Likewise, the reduction in e.o.p. of phage λ of groups V and VI, apparently very similar to that of group IV, has never been transferred to recipients. Hence group V is considered with group VIII, since the effects that can be transferred to recipient strains are those typical of the latter group. The one strain of group VI is considered with IX, although the transmissible effects are not identical to those of group IX (see chap. 3 section B.v). Similarly the reduction in e.o.p. of phage P1 by group VII has not been transferred by conjugation; the

effects which are transmitted are those typical of group X, so these two groups are considered together. Finally, strain J5-3 R132 has not been shown to transfer its reduced e.o.p. of phages P1 and P2 (see chap. 4, section I.B).

These non-transmissible reductions in e.o.p. are not directly relevant to the subject of this thesis, and while it is not intended to comment on the Enterobacteriaceae isolates from which the J5-3 strains were obtained, it is possible that these effects are relevant to methods of transfer between Enterobacteriaceae, or may be used to obtain information concerning the plasmids carried by the original strain. The strains which do not transfer some reductions in e.o.p. still carry sex factors since they do donate drug resistance determinants and sometimes other effects on phage plating. This fact reduces the possible explanations for the non-transmissible nature of the effects, and only two seem reasonably likely. One is that there was transfer of chromosomal material into the J5-3 recipient: R factors are known to transfer chromosomal genes at low frequency (Sugino & Hirota, 1962). Integration of the chromosomal genes is the most likely mechanism of their maintenance in the recipient. Alternatively, the original Enterobacteriaceae could have been lysogenic for a phage. Either transduction, with subsequent integration of the genes into the J5-3 chromosome, could have occurred; or if the determinants were on the phage genome, lysogenisation would have to be postulated. Although this latter necessitates postulating that either induction of the prophage, or transduction, was negligible under the conjugation conditions used in this thesis, it receives some support from the fact that a segregant of the group IV strain J5-3 R276 was found which did not reduce the

e.o.p. of phage λ, although continuing to be resistant to all the drugs to which the original J5-3 R276 was resistant.

The nature of the non-transmissible effects on phage plating is not known, but for groups IV, V and VI the e.o.p. of phage λ may well be reduced as a result of negligible burst size, whereas group III is of considerable interest in that the burst size is not significantly reduced and it is possible that this may be a case of restriction, only transmission coefficient being affected (table 3.8.)

iii The non-modifying groups

Out of the original ten R^+ strain groups, only six will donate distinct genetic determinants to recipient strains. Hence the R factors can be considered in six groups, of which two are characterised by host specificity systems (the hsI and hsII determinants) while the remaining four (groups VI, VIII, IX and X) do not modify the phages whose e.o.p. they lower. It is possible that such strains are restricting but not modifying, as is the wØ found by Kerszman, Glover & Aronovitch (1967), but other evidence must be considered before a conclusion can be reached.

Due to poor adsorption and lack of antisera, it was not possible to investigate the adsorption, transmission coefficient or burst size of the female specific phages. The only experiments performed were on lysis of phage T3 infected cultures (see Fig. II). Groups IX and X showed no significant deviation in lysis time from the control R^- culture, and no conclusion can be reached as to the reason for the slight reductions in e.o.p. of the female specific phages found with these two groups, though the abnormal plaque morphology is suggestive of either adsorption difficulties, or low burst size. Cultures of strains of groups VI and VIII, however, fail to lyse 60 min. after infection with phage T3, while the

control R⁻ culture lyses at about 30 min. Even though it is not possible to identify the step in phage infection which is affected, the continued growth of the infected cultures of groups VI and VIII suggests that no phage are synthesised, possibly that few or no phage functions are expressed in the infected cell. If the cells of groups VI and VIII do become infected with phage T3, then it is possible that this represents a case of restriction without modification. Unfortunately, as there is no data on adsorption of these phages, it is possible that the defect is prior to adsorption, and no further explanation need be sought.

Groups VIII and X plate BF23 with only a slightly reduced e.o.p., accompanied by a decrease in plaque size. There is no detectable difference from R⁻ control cultures in lysis time (Fig. I), adsorption, transmission coefficient, nor, for group X, burst size (table 3.15.). The burst size for a group VIII strain is consistently lower than for the R⁻ control culture (table 3.15.) and though the difference is too small to be conclusive it may be that the group VIII strains have a reduced e.o.p. of BF23 due to a reduced burst size. No conclusion can be drawn for group X.

Evidence shows that the low e.o.p. of phage BF23 on groups VI and IX is clearly related to the reduction in e.o.p. of phage BF23 by colIb⁺ strains, observed by Strobel & Nomura (1966). Mutants of phage BF23 selected for growth on any group IX strain or on the group VI strain, grow normally on all the group IX strains, group VI and on a colIb⁻ strain. Also, phage T5 had been reported by Nisioka & Ozeki (1968) to have a reduced e.o.p. on colIb⁺ strains and, when tested, this phage was found to have a reduced e.o.p. on all group VI and IX strains. Nisioka and Ozeki (1968) had found

early lysis of phage BF23 infected colIb$^+$ cultures, and this was likewise observed for cultures of strains of groups VI and IX. Adsorption of phage BF23 to these strains is not affected, but transmission coefficient is reduced 10 to 20 fold and burst size is probably negligible (table 3.15.). This agrees with the findings of Strobel and Nomura (1966), that the colIb$^+$ strains do not produce viable phage after phage BF23 infection, but do lyse. With all this evidence, it was not surprising to find that all except one of the group IX strains are colIb$^+$. As they were not further investigated, it is not possible to say whether the col factor is existing as an independent plasmid or is associated with the drug resistance determinants. However, a strain carrying R62 has been investigated by Romero & Meynell (1969) and was found to carry the colI character associated with an fi$^+$ R factor which also determined the I-type pilus.

Group VI, however, is not colI$^+$, in fact does not produce any detectable colicin, but is resistant to colicin Ib. This R factor may be one of those found by Siccardi (1966) to reduce the e.o.p. of BF23, but while being col$^-$ were resistant to colicin Ib. The remaining group IX strain, J5-3 R330, is neither colIb$^+$ nor colicin Ib resistant. It is possible that while genetic determinants associated with colIb can disrupt the function of the phage BF23 in the infected cell, these determinants can also be associated with other transfer factors, and are not the exclusive property of either colIb$^+$ or colicin Ib resistant strains. It is also possible that strains such as J5-3R252 have lost the ability to produce colicin Ib while retaining the immunity to it, i.e. contain colIb mutants. Although Strobel & Nomura (1966) found no breakdown of radioactive DNA to acid soluble fragments when they

infected a colIb* culture with labelled phage BF23, this does not disprove the possibility that this phenomenon is a case of restriction without modification. It is noteworthy that the groups which reduce the e.o.p. of phage BF23 only slightly (groups VIII and X) do not produce, nor are resistant to, colicin Ib. Another, possibly minor, point, is that a reduction in e.o.p. of phage BF23 is always accompanied by a reduction in e.o.p. of the female specific phages, and vice versa. The reductions in e.o.p. may be both large (groups VI and VIII), both slight (group X), or one large and the other slight (group IX).

The investigations of the non-modifying groups has not in any instance revealed the precise nature of the lesion in phage infection which is occurring, but at least two groups might be restricting but not modifying the phages involved, these being group VIII and the group(s) associated with colIb group IX(and group VI).

iv The modifying groups

Twelve R* strains were found which both restricted and modified the phages λ, ø80, P1, P2 and T1. Of these, eleven form one group, group II,which appears to have been isolated previously since the R15 of Watanabe (Watanabe et al., 1964b) has the same host specificity. All the group II strains actually contain fi- R factors which are associated with the hsII determinants (see chap. 4 section I.B for explanation of the apparent anomalies). Group I is represented by only one R* strain, J5-3 R124, and as R124 is fi*, this is the only recorded fi* R factor determining restriction and modification.

Both of these two groups determine host specificites resembling that of phage P1 rather than the chromosomal K and B host specificities in that the phage T1 is restricted by them. In all

other ways these appear to be two typical host specificity systems, and they will be the subject of the remaining sections of this thesis.

CHAPTER 4

INVESTIGATION OF THE R* STRAINS, AND CONSTITUENT R FACTORS, OF GROUPS I AND II

In chap. 3 it was stated that when an R* strain of group II was used as donor, it was possible to isolate ex-conjugants carrying the hsII determinants in addition to the resistance determinants. When, however, the strain J5-3 R313 was used as the donor, it was noted that not all ex-conjugants selected for Sm resistance carried the hsII determinants. The strain J5-3 R313 carried determinants for resistance to the drugs Tc, Sm and Su, as well as for hsII; all of these were transmissible. The presence of an fi⁻ R factor can be masked by the presence of an fi* R factor in the same strain, if repression of F is the only character tested, and strains in which two R factors co-exist are not uncommon (Romero and Meynell, 1969). However, the R factor R313 had been previously reported to be fi⁻ (Lawn et al., 1967) and the strain J5-3 R313 was checked to behave as fi⁻. As there was no previously recorded case of two fi⁻ R factors co-existing in the same cell, the transmissible genetic determinants in the strain J5-3 R313 were further investigated. As a result of this study, five other group II strains and the group I strain were also investigated.

The method of transfer of drug resistance used throughout chapter 4 is that detailed in chap. 2 section O.i.

SECTION I

TRANSFER OF RESISTANCE AND HOST SPECIFICITY DETERMINANTS FROM R$^+$ STRAINS OF GROUPS I AND II

A. Transfer of Resistance and hsII Determinants from Strains carrying R313

If the strain J5-3 R313 carried more than one plasmid, separation of the plasmids might be observed by scoring determinants transferred when each drug resistance carried by the strain is selected for separately. The strain J5-3 R313 was therefore used as a donor in crosses in which the recipient was C600: the results of two experiments are presented in table 4.1. Selection for resistance to either Sm or Su showed the same result: some colonies were resistant to Sm and Su only, others were resistant to Tc as well. The proportion of the two types of ex-conjugant was variable from experiment to experiment, 10 to 50% of the colonies resistant to Sm and Su were also Tc resistant, but in each experiment the proportion of colonies resistant to Tc, Sm and Su was similar irrespective of whether Sm or Su was used as the selective agent (columns three and four in table 4.1.). In these experiments, none of the colonies selected for resistance to Tc showed resistance to either Sm or Su (fifth line, table 4.1.), and separation of the resistances to Sm and Su was never observed. The results of the tests for restriction of λ.K by the different classes of recombinants showed a clear association of the hsII determinant with the Tc resistance determinant; all Tc resistant colonies, however selected, were hsII$^+$, while ex-conjugants resistant to only Sm and Su did not restrict λ.K (column five, table 4.1.).

Three types of drug resistant ex-conjugant had thus been found: resistant to Tc and hsII*; resistant to Tc, Sm and Su, and hsII*; resistant to Sm and Su. An example of each type of colony was purified (see chap. 2 section O.ii) and shown to carry a sex factor by the ability to transfer the drug resistances carried (tables 4.2, 4.10. and 4.11.). The two derivative strains carrying fewer determinants than the original J5-3 R313 were named according to the designations given to the assumed segregant R factors they carried. The R factor carrying determinants for Tc resistance and hsII was termed R313-T-3, and the R factor carrying Sm and Su resistance determinants was called R313-SS-1 (table 2.3.). When all the sex factor associated determinants of the original J5-3 R313 are present in a strain it is, for simplicity, said to carry R313.

The strain C600 R313 behaved as a donor in an identical manner to J5-3 R313 in that three types of ex-conjugant were isolated: Sm resistant; Tc and Sm resistant and also hsII*; Tc resistant and hsII* (table 4.2., resistance to Su was not scored in this experiment). In this experiment colonies selected for resistance to Tc were found which had also acquired Sm resistance. Clearly transfer of Sm resistance is rarer than transfer of Tc resistance - no Sm resistant colonies had been found after selection for Tc resistance using J5-3 R313 as donor (table 4.1.).

It was concluded from these experiments that the strain J5-3 R313 contained two different plasmids, one determining resistance to Sm and Su, the other carrying determinants for Tc resistance and hsII. Both could be associated with, or were part of, a sex factor.

Table 4.1.

Drug resistance patterns of, and restriction of λ.K by, ex-conjugants from the cross J5-3 R313 X C600

Selection	Drug resistance patterns found	No. of colonies found		Restriction* of λ.K
		Expt. 1	Expt. 2	
Sm	Sm Su	71	98	-
	Tc Sm Su	48	30	+
Su	Sm Su	42	104	-
	Tc Sm Su	65	17	+
Tc	Tc	75	68	+

* A minimum of ten colonies of each type tested for restriction, by method of chap. 2 section G.ii.d.

Table 4.2.

Drug resistance patterns of, and restriction of λ.K by, ex-conjugants from the cross C600 R313 X J5-3

Selection	Drug resistance patterns	No. of colonies found	Restriction* of λ.K
Tc	Tc	109	+
	Tc Sm	3	+
Sm	Sm	67	-
	Tc Sm	67	+

* A minimum of ten colonies of each type tested for restriction

B. Transfer of Genetic Determinants from the Group II Strains J5-3 R132, J5-3 R250, J5-3 R269, J5-3 R270, and J5-3 R348

All group II strains were Tc resistant, and five were resistant only to Tc (J5-3 R199, J5-3 R203, J5-3 R204, J5-3 R245, J5-3 R265). One of the remaining strains, J5-3 R313, appeared to segregate the determinants for Tc resistance and hsII from the determinants for resistance to Sm and Su in conjugation experiments (chap. 4 section I.A.). The remaining five strains were therefore used as donors in crosses to C600, and the drug resistant ex-conjugants were analysed to see if the determinants for Tc resistance and hsII could be separated. Since two of the strains were also those scoring as fi$^+$, and these two were the only exceptions to the association of hsII with fi$^-$ R factors, the drug resistant ex-conjugants were of especial interest as they could be tested for fi character if more than one type of ex-conjugant was observed per strain.

The results of the analysis of the crosses are presented in table 4.3., and from these results a summary of the types of drug resistant ex-conjugants can be made (table 4.4.). The resistance to Su is indicated in brackets, thus (Su), where the inability to screen for Su resistance by replica plating led to an ambiguity, since the available data did not exclude the existence of two types of ex-conjugant, the Su sensitive and the Su resistant, but the type listed was found when resistance to Su was selected for.

Only in the case of R132 were the determinants for Tc resistance and for hsII ever separated, but when this strain was used as a donor, ex-conjugants were found which were hsII$^+$ and resistant to Sm and Su, but not to Tc. The status of the Tc resistance in the original J5-3 R132 was not explored further, but

Table 4.3.

Patterns of transfer of drug resistance and HsII locus from J5-3RII+ strains to C600

R factor	Drugs carried	Selection	Drug resistances found *	No. of colonies found Expt.1	No. of colonies found Expt.2	Restriction of phage λ.K**
R132	Tc Sm Su	Sm	Sm Su	11	2	+
			Tc Sm Su	14	3	+
		Su	Tc Sm Su		2	+
R250	Tc Su	Su	Tc Su	144	167	+
			Su	0	3	-
R269	Tc Sm Su Km	Tc	Tc Sm	97	35	+
			Tc Sm Km	30	117	+
		Sm	Tc Sm	15	2	+
			Tc Sm Km	40	46	+
			Sm Km	15	96	-
		Su	Tc Sm Su	0	2	+
			Tc Sm Su Km	2	39	+
			Sm Su Km	128	102	-
		Km	Tc Sm Km	25	36	+
			Sm Km	120	99	-

Table 4.3. (cont'd)

R factor	Drugs carried	Selection	Drug resistances found *	No. of colonies found		Restriction of phage λ.K**
				Expt.1	Expt.2	
R270	Tc Sm Su	Tc	Tc Sm	159	80	+
		Sm	Tc Sm	153	105	+
			Sm	3	0	+
		Su	Tc Sm Su		35	+
			Su		118	-
R348	Tc Sm Km Cm	Tc	Tc Sm	16	112	+
			Tc Km Cm	97	27	-
			Tc Sm Km Cm	8	2	+
		Sm	Tc Sm	75	68	+
		Km	Tc Km Cm	109	33	-
			Tc Sm Km Cm	19	4	+
		Cm	Tc Km Cm	123	51	-
			Tc Sm Km Cm	33	3	+

* Su resistance is only scored in the case of R132 where there were so few colonies as a result of the low transfer of this factor that they were all streaked to test for Su resistance.

** At least 20 colonies of each type (if available) tested.

Table 4.4.

Determinants carried by ex-conjugants from the crosses presented in Table 4.7.

Donor R factor	Types of ex-conjugant found
R132	Sm Su hsII
	Tc Sm Su hsII
R250	Tc Su hsII
	Su
R269	Tc Sm (Su) hsII
	Tc Sm (Su) Km hsII
	Sm (Su) Km
R270	Tc Sm (Su) hsII
	Su
R348	Tc Sm Km Cm hsII
	Tc Sm hsII
	Tc Km Cm

Table 4.5.

MS2 plating and presumed fi character of the R factor of R^+ F^+ strains

R factor	MS2 plating	No. of colonies tested	Presumed fi character
R269-TSS-1	+	3	-
R269-SSK-1	-	6	+
R348-TS-1	+	3	-
R348-TSKC-1	-	7	+

Tc sensitive segregants of the original strains have been found, and the Tc resistance was clearly on a plasmid. It was also discovered that C600 derivatives of R132 were now sensitive to the phages P1 and P2, and restricted and modified these phages with normal group II host specificity. The original J5-3 R132 had apparently been resistant to phages P1 and P2.

Although ex-conjugants selected for Tc resistance could not be scored *en masse* for resistance to Su, amongst a few streaked out from the cross with J5-3 R250 as donor, both Tc resistant hsII$^+$ colonies, and colonies resistant to both Tc and Su, and carrying the hsII determinant were found. In the case of the strain J5-3 R250 it is therefore clear that the hsII and Tc resistance determinants segregate together, and can be separated from the determinant for Su resistance.

When the donor strain was J5-3 R270, the determinants for resistance to Tc and Sm, and hsII were not separated, but the determinant for resistance to Su segregated from the other determinants. Again there was a close association of the determinants for Tc resistance and hsII.

When the donor strains were J5-3 R269 and J5-3 R348, the two strains which were scoring as *fi*$^+$, essentially similar results were obtained - three types of drug resistant ex-conjugant were found. If it was assumed that two R factors were present in the original strains, the three ex-conjugant types could be explained as the presence of each R factor separately and the presence of both R factors (as in the original strain). In both cases the hsII determinant was associated with a determinant for Tc resistance. Four C600 strains carrying the presumptive R factors derived from

J5-3 R269 and J5-3 R348 were further purified (see chap. 2 section O.ii). The presumptive R factors were given the designations R269-TSS-1, R269-SSK-1, R348-TS-1 and R348-TSKC-1 (table 2.4), the R269-TSS-1 and R348-TS-1 being the R factors associated with the hsII determinants. These four strains were used as donors for the preparation of J5-3 R^+F^+ strains (see chap. 2 section O.ii), which were checked to be F^+ on the basis of the lowering of the e.o.p. of phage T3. The results of testing the ability of the J5-3 R^+F^+ strains to plate MS2 is presented in table 4.5. It is seen that the R factors associated with the hsII determinants, R269-TSS-1 and R348-TS-1, are both $\underline{fi}^-$, and the second R factor which had been present in the original strain was $\underline{fi}^+$. The transfer of the resistance determinants to the J5-3 F^+ strain had also demonstrated the presence of a sex factor in each of the four derivative C600 strains.

Although the apparant association of hsII and Tc resistance determinants failed to hold, in ten out of the eleven group II strains the hsII determinant was associated with Tc resistance determinants. The two apparently $\underline{fi}^+$ group II strains proved to carry masked $\underline{fi}^-$ R factors which were the R factors associated with the hsII determinants, so there is no known $\underline{fi}^+$ R factor associated with hsII.

C. Donor Ability of Strain J5-3 R124 for Tc Resistance and the hsI Determinants

There is a close but not complete correlation between Tc resistance and the hsII determinant, and all group II strains have the hsII determinant associated with an $\underline{fi}^-$ R factor. But the group II strains were also frequently complex, sometimes clearly

harbouring two R factors, an fi+ and an fi-, in other cases harbouring either two fi- R factors, or an R factor and a plasmid. The status of the hsI determinant in the only group I strain, J5-3 R124, was therefore investigated. Since it is not possible to efficiently select hsI+ ex-conjugants, only selection for resistance to Tc could be applied after conjugation.

The results of three experiments in which R124 was used as a donor of Tc resistance to three different recipients; a K strain, C600; a B strain, Btrp-; and a C strain, C thr-, are presented in table 4.6. No segregation of the hsI and Tc resistance determinants was observed in these experiments, nor in the preparation of any other strain carrying R124. Table 4.6. also illustrates the fact that R124 is subject to restriction by B type host specificity, the frequency of transfer of R124 to the B strain being a hundred fold less than to either the K or C strain. Three strains carrying R124, a C600, a B and a C strain, were purified (chap. 2 section O.ii) and kept as stock strains for use in other experiments (chap. 5).

Table 4.6.

Transfer of Tc resistance and hsI to

K, B and C recipients from the donor strain J5-3 R124

Recipient strain	Transfer frequency of Tc resistance	No. of Tc resistant colonies tested	No. of colonies hsI+ *
C600	7×10^{-4}	31	31
B trp-	9×10^{-6}	13	13
C thr-	5×10^{-4}	17	17

* Tested for restriction of the appropriate phages λ.K, λ.B and λ.C by the method of chap. 2 section G.ii.c.

D. Discussion

i The segregation of determinants of the original J5-3 R313

Repeated mention has been made of the fact that the survey results presented in chapter 3 were obtained on R^+ strains which had been subcultured from stock slopes, and without further investigation there is no guarantee that the plasmids carried are those carried by other strains nominally bearing the same R factor. However, the group II strain J5-3 R313 appeared to be behaving as the R313 strain described by Lawn et al. (1967) in that it was fi$^-$ and carried determinants for resistance to Tc, Sm and Su. It was therefore unexpected that loss of the Tc resistance and hsII determinants should sometimes occur on transfer of the Sm and Su resistance determinants by conjugation. The experiments in section I.A of this chapter showed clearly that the Sm and Su resistance determinants were closely associated, as were the Tc resistance and hsII determinants. The establishment of these associations allowed these two sets of characters to be thought of as two linkage groups, capable of independent transfer, either group of determinants being associated with sex factor activity after segregation.

The strain J5-3 R313 had simply been envisaged as carrying a single R factor, a sex factor associated with determinants for resistance to Tc, Sm and Su and for hsII. This idea was no longer tenable. Even if the various determinants acted as a replicon (and there is no evidence for this), they were clearly separable into two linkage groups by conjugation. Hence the strain carries a minimum of two plasmids. Stable co-existence of two or more plasmids in one cell is known, e.g. F and an R factor (Sugino & Hirota, 1962) and in the case where there are two transmissible plasmids

(sex factors) in the same strain, it is probable that transfer occurs independently. This is the situation when strains carry two R factors, an fi⁻ and an fi⁺, the R factors transfer independently but a recipient can receive both (Romero & Meynell, 1969). When a transmissible and a non-transmissible plasmid co-exist in the strain, as when the transmissible colI is present with colE1 in an E. coli strain, transfer of the one lacking sex factor activity (colE1) requires concomitent transfer of the sex factor (colI), but the sex factor may transfer on its own (Monk, 1964).

The results obtained with J5-3 R313 (table 4.1.) indicated that co-transfer of the two linkage groups was frequent when selection was for Sm or Su resistance, rare when Tc resistance is selected for, but in either case independent transfer of the two linkage groups occurred. This was therefore like the known cases of two plasmids with sex factor ability being present in the same strain, and did not bear any resemblance to the situation encountered with colI and colE1. The likelihood of two R factors being present was increased by the fact that both segregant types of ex-conjugant retained sex factor ability, the transfer frequencies from segregant strains being the same as from the parent strain (see tables 4.10. and 4.11.).

The C600 ex-conjugant receiving all the known transmissible determinants of the original J5-3 R313 showed the same type of segregation pattern when it was used as a donor (table 4.2.), so that it was concluded that co-transfer of the determinant linkage groups was not host dependent and did not involve any permanent attachment of the linkage groups.

The simplest explanation of the behaviour of J5-3 R313 was

to consider it as harbouring two R factors, one carrying the Tc resistance and hsII determinants, the other the Sm and Su resistance determinants. The strain J5-3 R313, however, does not contain any determinants suppressing the plating of MS2 on F^+ strains, nor has any derivative been shown to be fi$^+$ (table 4.12.). Hence both R factors would be fi$^-$, and as the co-existence of two fi$^-$ R factors has not been previously reported, except when this is forced by drug selection, it was decided to search for further evidence, and consider other possibilities, before accepting this solution.

The possibility was also considered that R313 was a single R factor from which the spontaneous loss of some determinants was high. It is unlikely that Tc resistance and hsII character are determined by the same set of genes (linkage is discussed in relation to the entire group II in section D.ii of this chapter), and though it is possible that Sm and Su resistance could be determined by a single gene altering, perhaps, cell wall properties and therefore adsorption, segregation would have had to involve a large deletion, or an event similar to excission of a prophage, or reversion of an Hfr to F^+ mating type. Though such an event may occur, it did not seem likely that it was the explanation of the behaviour of R313 carrying strains. The data available (tables 4.1. and 4.2.) could only be explained if half of the Sm and Su resistant cells had lost Tc resistance, and less than 1% of Tc resistant cells were still Sm and Su resistant. This was not the case as far as the whole population was concerned (see section II.A of this chapter). It could be argued that the R factor segregated within the cell, without loss of the resulting non-transmissible plasmid from the cell, but data on transduction of the resistance determinants which makes this unlikely, will be presented

in section II of this chapter with further studies on R313.

ii The group II strains

The discovery that J5-3 R313 behaved in a manner explicable by its carrying two *fi*⁻ R factors revived interest in the other group II strains, and two possible relationships were explored. One was based on the observation that all the group II strains, and the one group I strain, were Tc resistant. Of the 11 group II strains, five were resistant to Tc only, and one displayed a separation of the hsII and Tc resistance determinants from the determinants for Sm and Su resistance. Hence it was of interest to see if the Tc determinant was always associated with the hsII determinant in the remaining five group II strains. The other relationship was that of *fi* character and the hsII determinants. Three previous reports exist of the restriction and modification of coliphages. Yoshikawa and Akiba (1962) found three *fi*⁻ R factors restricting phages λ and P1, and definitely modifying phage λ. The restriction levels of these strains suggested that they could be group II. Molina (1964) reported an R factor which restricted and modified phage T1, and was also reported to restrict phage P1 (Molina *et al.*, 1965). Again this might have been either group I or group II, or entirely different, but the data was compatible with it being group II and *fi*⁻. The third report, by Watanabe *et al.*,(1964b) was of two *fi*⁻ R factors restricting and modifying several phages, including phages λ and T1, and here it was possible to check that the host specificity was of the hsII type, since one of the R factors, R15, was made available for this purpose (both R factors had the same specificity). The *fi* character of all the group II strains was rechecked, and only J5-3 R269 and J5-3 R348, two of the strains already chosen for

investigation, were *fi*⁺. Hence these were the only two group II strains known to carry *fi*⁺ R factors, and the only other R factor definitely reported to restrict coliphages and be *fi*⁺ is the group I strain R factor, R124.

The association of Tc resistance and hsII proved not to be universal, though only one of the total eleven group II strains showed a separation of the two determinants. J5-3 R132 was a Tc, Sm and Su resistant strain, which showed frequent loss of Tc resistance, and it was clear that after transfer the Tc resistance is not associated with the hsII determinants, but the Sm and Su resistance determinants seemed to be associated with the hsII determinants. No Tc resistant ex-conjugants were ever observed which did not carry Sm and Su resistance as well, so it is possible that the Tc resistance determinants are on a separate, non-transmissible plasmid, but as the transfer frequency from J5-3 R132 is low, few colonies were scored. The strain J5-3 R250 was difficult to use, because it is resistant only to Tc and Su. The hsII determinant is clearly linked with the Tc resistance determinant, and while the occasional colony resistant only to Su was found, these were rare. Hence it is not clear whether there is more than one plasmid, and if so, whether both are transmissible or not. When the strain J5-3 R270 was used as a donor, the hsII, and Tc and Sm resistance determinants were all transferred together. The only evidence to suggest that this was not a single R factor was the isolation of a considerable number of colonies resistant only to Su (and hsII⁻) in one experiment.

The two apparently *fi*⁺ strains were both shown to carry an *fi*⁺ and an *fi*⁻ R factor. There is no need to postulate the presence

of any other plasmid in the strains J5-3 R269 and J5-3 R348 than the two R factors found to be carried in each strain. In both cases the R factor associated with the hsII determinant carried resistance to Tc and was *fi*⁻. Hence there is now no known case of an *fi*⁺ R factor associated with hsII determinants.

The frequency with which Tc resistance and hsII were found to be associated in this sample of R factors may have been a coincidence. Lebek (1969) reported that over 70% of drug resistant isolates of *Enterobacteriaceae* were resistant to Tc, either alone or combined with resistances to other drugs. The apparent association of host specificity and Tc resistance determinants may merely reflect the normal high incidence of Tc resistance.

It is not so easy to suggest a rationale for the association of hsII with *fi*⁻ R factors only. Perhaps the origin of the hsII determinants was a chromosome or plasmid which had homology for *fi*⁻ but not *fi*⁺ sex factors. This association of *fi*⁻ R factors and hsII determinants was not only a feature of this particular survey, but was previously observed by Watanabe *et al.* (1964b) and could hold for the other two reports of presumptive hsII type restriction and modification.

Another possibly interesting, but unexplained feature is the high incidence of the hsII type of host specificity. The R124 described here is the only other R factor associated with a different host specificity which has been clearly defined.

iii *The group I strain*

As J5-3 R124 was only resistant to Tc, analysis of this strain was limited to selection for Tc resistance, and scoring the restriction of phage λ on the Tc resistant ex-conjugants. No evidence of separation of the two determinants was obtained and it

was concluded that R124 is a "simple" R factor, i.e. that only one R factor was present in the strain.

The restriction of R124 by B was incidentally noted in these experiments.

SECTION II

FURTHER ANALYSIS OF THE R FACTOR ORIGINALLY TERMED R313

Evidence was being sought to prove or disprove the hypothesis that the original strain J5-3 R313 carried two fi⁻ R factors. Two methods of investigation suggested themselves; selection of spontaneous drug sensitive segregants, and phage P1 transduction.

A. Spontaneous Segregants of J5-3 R313 Sensitive to One or More Drugs

The conjugation experiments presented in section I of this chapter bias the interpretation of the results obtained in favour of the presence of two R factors, since ex-conjugants receiving drug resistance are likely to have also received a sex factor. Selection of spontaneous drug sensitive segregants of the J5-3 R313 would not be open to this criticism. Since screening of colonies of J5-3 R313 failed to reveal any drug sensitive segregants, it was assumed that the normal segregation rate was less than 1%, and that a preliminary enrichment before screening was necessary. Penicillin selection (chap. 2 section N) was used to obtain segregants sensitive to the bacteriostatic drugs Tc and Su, though it could not be used to obtain segregants sensitive to Sm, which is bacteriocidal.

The yield of drug sensitive segregants remained low, but the results, presented in table 4.7., were unambiguous. The loss of

Table 4.7.

Drug sensitive segregants of J5-3 R313

found after penicillin selection

Sensitivity selected for	Lost drug resistance	Retained drug resistance	Restriction and modification*	No. of colonies tested
Tc	Tc	Sm Su	-	2
Su	Sm Su	Tc	hsII	5

* tested with the phages λ, ø80, P1 and P2, all K grown.

Table 4.8.

Drug resistance and host specificity

of C600 transduced by phage P1 grown on strain J5-3 R313

Selection	Drug resistance of transductants	Restriction* of phage λ.K	No. of colonies tested	
			Expt. 1	Expt. 2
Tc	Tc	+	123	20
Sm	Sm Su	-	25	19
Su	Sm Su	-	6	28

* Tested by the method of chap. 2 section G.ii.c.

Table 4.9.

Failure of Sm resistant transductants of

C600 to transfer Sm resistance

Source of P1 lysate	No. of colonies transferring Sm resistance at $< 10^{-6}$	No. of colonies transferring Sm resistance at $> 10^{-6}$
J5-3 R313	20	0
J5-3 R313-SS-1	12	0

Table 4.10.

Average transfer frequencies observed for C600 R^+ donors to J5-3 recipients when R is R313 or an R313 derivative

R factor	Transfer frequency/donor selecting resistance to:		
	Tc	Sm	Su
R313	9×10^{-4}	3×10^{-3}	3×10^{-3}
R313-T-1	2×10^{-3}	-	-
R313-T-2	1×10^{-3}	-	-
R313-T-3	1×10^{-3}	-	-
R313-T-4	1×10^{-3}	-	-
R313-SS-1	-	4×10^{-3}	3×10^{-3}
R313-SS-2	-	1×10^{-3}	1×10^{-3}
R313-SS-3	-	$< 10^{-6}$	$< 10^{-6}$
R313-SS-4	-	$<10^{-6}$	$<10^{-6}$
R313-SS-5	-	$<10^{-6}$	$<10^{-6}$
R313-SS-6	-	$<10^{-6}$	$<10^{-6}$

Table 4.11.

Average transfer frequencies observed for J5-3 R^+ donors to C600 recipients when R is R313 or an R313 derivative

R factor	Transfer frequency/donor selecting resistance to:		
	Tc	Sm	Su
R313	3×10^{-4}	1×10^{-4}	3×10^{-4}
R313-T-1	4×10^{-5}	-	-
R313-SS-1	-	3×10^{-3}	5×10^{-3}
R313-SS-2	-	3×10^{-3}	4×10^{-3}

Tc resistance is accompanied by the loss of the hsII determinants, but Sm and Su resistance determinants are retained; whereas the loss of Su resistance is accompanied by loss of Sm resistance, but retention of Tc resistance and hsII by the cell. One colony of each type was further purified, the R factors given the titles R313-T-1 and R313-SS-2 (table 2.4.) and shown to be transmissible (tables 4.10. and 4.11.) and hence to retain a sex factor.

B. Phage P1 Transduction and Investigation of Transductants

i Transduction with phage P1 grown on J5-3 R^+ strains

Phage P1 generally transduces the entire R factor; co-transduction of drug resistances and the sex factor activity is used as evidence of a single structural unit for the R factor (Mitsuhashi et al., 1962). The strain J5-3 R313 was investigated by growing phage P1 on it, and using the lysate to transduce C600 by the method detailed in chap. 2 section M. The results of analysis of drug resistant transductants is presented in table 4.8. As was by then expected, transductants selected for resistance to either Sm or Su were resistant to both, but were sensitive to Tc and were not $hsII^+$. Transductants selected for resistance to Tc were only resistant to Tc, but carried the hsII determinants. A total of six transductants, one for each drug resistance from the two experiments were purified and carefully checked for the hsII restriction and modification, confirming the result first obtained. The presumptive R factors were named by adding the suffixes -T-2, -T-4, -SS-3, -SS-4, --S-5 and -SS-6 to R313 (table 2.4.). The transfer properties of the two classes of transductant were, however, very different. The Tc resistant transductants transferred the Tc resistance at a similar

frequency to R313 in C600, but the Sm and Su resistant transductants could not be shown to transfer resistance to either drug; the level of transfer was very much less than 10^{-6} drug resistant ex-conjugants per donor (table 4.10.).

The transductions were repeated with a newly prepared stock of phage P1 J5-3 R313, and with a lysate made from the strain J5-3 R313-SS-1; Sm and Su resistant colonies obtained from these transductions were tested for the ability to transfer Sm resistance to a J5-3 recipient. The results in table 4.9. showed that none of the transductants from these experiments were able to transfer Sm resistance at a frequency greater than 10^{-6}/donor. Thus if co-transduction of Sm and Su resistance and sex factor ability occurs, such transductants are rare, and this is not solely a feature of the original strain, but of a strain carrying the derivative R factor R313-SS-1 as well.

ii Mobilisation of the non-transmissible Sm and Su resistance of transductants

Plasmids which are essentially non-transmissible can sometimes be mobilised by the presence of a sex factor, e.g. colE1 can be mobilised by colI (Monk, 1964), and a non-transmissible resistance determinant was found to recombine with F to form a transmissible plasmid (Harada et al., 1964). An attempt was therefore made to mobilise the non-transmissible Sm and Su resistance of phage P1 transductants by using a number of sex factors: F; a known fi⁻ R factor, R143; a known fi⁺ R factor, R124; a Tc resistant segregant of R313, R313-T-1. The last of these, as well as Sm and Su resistant derivatives of R313, were checked to be fi⁻ (table 4.12.). Since the transduced Sm and Su resistance determinants were not trans-

missible, the strains, listed in table 4.13. with the presumed plasmid associated determinants detailed, were made using the transductants as the recipient strains. Two strains were used as recipients, one selected for Sm resistance, C600 R313-SS-3, and one selected for Su resistance, C600 R313-SS-4, though no difference was found in the behaviour of the two. The conjugation method was as in chap. 2 section O.ii and iii. During preparation of the strains, no evidence of superinfection immunity was observed, though no accurate figures were obtained.

The strains listed in table 4.13. were used as donors to J5-3 recipients, and the results of the crosses (method of chap. 2 section O.i) are presented in table 4.14. Transfer of the R factors R124, R143 and R313-T-1 occurred at about 10^{-4}/donor for R124, and 10^{-3}/donor for the other two. The transfer of F was not measured. Only in the case of the strains carrying F or R143 was any transfer of the Sm resistance observed, and only in the case of the R143 did it approach the frequency with which the sex factor was transferring.

An analysis was made of some ex-conjugants, and the results are in table 4.15. For R124, this analysis confirmed the finding that the Sm resistance determinant is not transferred with the Tc resistance determinant, all Tc resistant colonies were resistant to Tc only. In the case of R143, the difference in transfer frequency of the two drug resistances Sm and Km, was reflected in the fact that Km resistant ex-conjugants only rarely carry Sm resistance, whereas it is the exceptional Sm selected colony which does carry Km resistance (only 3 out of the 80 colonies tested).

A total of 16 Sm resistant colonies from the crosses where the donors carried F were tested by the criterion of both MS2 plating, and

Table 4.12.

MS2 plating and presumed fi character of F+ strains carrying a segregant R factor

R factor	MS2 plating	No. of colonies tested	Presumed fi character
R313-SS-1	+	4	-
R313-SS-2	+	4	-
R313-SS-3	+	9	-
R313-SS-4	+	7	-
R313-T-1	+	5	-

Table 4.13.

R factor associated drug resistance and host specificity of C600 R313-SS-3 and C600 R313-SS-4 carrying a sex factor

Plasmids and sex factors carried	Drug resistances	Host specificity*
R313-SS-3 and F	Sm Su	-
R313-SS-4 and F	Sm Su	-
R313-SS-3 and R124	Tc Sm Su	hsI
R313-SS-4 and R124	Tc Sm Su	hsI
R313-SS-3 and R143	Sm Su Km	-
R313-SS-4 and R143	Sm Su Km	-
R313-SS-3 and R313-T-1	Tc Sm Su	hsII
R313-SS-4 and R313-T-1	Tc Sm Su	hsII

* Tested for restriction with phage λ.K by the method of chap. 2 section G.ii.a, and modification as in chap. 2 section H.ii.

Table 4.14.

Average frequencies of transfer of strains carrying a P1 transduced Sm resistance determinant and a sex factor

Plasmids carried	Average frequency of transfer/donor of resistance to the drug			
	Tc	Sm	Su	Km
R313-SS-3 and F	-	1×10^{-6}	NS	-
R313-SS-4 and F	-	2×10^{-6}	NS	-
R313-SS-3 and R124	5×10^{-4}	$< 10^{-7}$	NS	-
R313-SS-4 and R124	1×10^{-4}	$< 10^{-7}$	NS	-
R313-SS-3 and R143	-	1×10^{-5}	NS	1×10^{-3}
R313-SS-4 and R143	-	4×10^{-5}	NS	2×10^{-3}
R313-SS-3 and R313-T-1	2×10^{-3}	$< 10^{-6}$	$< 10^{-6}$	-
R313-SS-4 and R313-T-1	3×10^{-3}	$< 10^{-6}$	$< 10^{-6}$	-

NS indicates not scored

Table 4.15.

Analysis of drug resistant ex-conjugants from four crosses

Plasmids carried by donor strain	Selection	Drug resistances	No. of colonies found Expt. 1	Expt. 2
R313-SS-3 and R124	Tc	Tc	50	70
R313-SS-4 and R124	Tc	Tc	54	
R313-SS-3 and R143	Sm	Sm	2	
		Sm Km	28	
	Km	Sm Km	0	2
		Km	52	38
R313-SS-4 and R143	Sm	Sm	1	
		Sm Km	49	
	Km	Sm Km	1	0
		Km	34	72

Table 4.16.

Transfer frequency of derivatives of C600 R313-SS-3 f^+ and C600 R313-SS-4 F^+

Plasmids believed to be carried	Frequency of transfer of Sm/donor Expt. 1	Expt. 2
R313-SS-3 and F	2×10^{-6}	3×10^{-6}
R313-SS-4 and F	1×10^{-6}	2×10^{-6}
R313-SS-3	$< 10^{-6}$	$< 10^{-6}$
R313-SS-4	$< 10^{-6}$	$< 10^{-6}$

reduction of the e.o.p. of T3, for the presence of F. Of the 16 colonies, 14 carried F, two did not. The F^+ colonies serve as donors of Sm resistance, but still at the low level observed for the parent strain, whereas no transfer of Sm resistance was observed for the colonies found to be F^- (table 4.16.).

C. Discussion

In section II of this chapter, evidence was sought to supplement the evidence of section I, and prove or disprove the hypothesis that J5-3 R313 had carried two *fi*$^-$ R factors.

The first method chosen for further investigation of J5-3 R313 was simply to search for segregants which were sensitive to one or more drugs to which the original strain had been resistant. In this way no selection pressure for the presence of a sex factor would be applied. The results, however, merely confirmed the findings of section I.A. of this chapter, since loss of Tc resistance is accompanied by the loss of hsII, the remaining Sm and Su resistances being transmissible, and loss of Su resistance also resulted in loss of Sm resistance, but left sex factor activity and the determinants for Tc resistance and hsII.

At this stage another possible explanation for the behaviour of J5-3 R313 was considered: that the strain carried the two linked groups of determinants, and a sex factor separate from either of the other two plasmids. Selection for transfer of a drug resistance would automatically select for transfer of the sex factor as well, assuming that the transfer of the drug resistance determinants resembled that of *colE1* in the presence of *colI* (Monk, 1964). Phage P1 transduction was therefore used for further investigation of the strain, since phage P1 is believed to co-transduce only those R

factor carried genes which are literally on a single R factor. This method has been used successfully for the separation of fi⁻ and fi⁺ R factors (Romero & Meynell, 1969).

Once again, the determinants carried in strain J5-3 R313 were seen to segregate into two groups, Tc resistance and hsII determinants, and Sm and Su resistance determinants. There was no evidence for joint transduction of any other pair of determinants of these four. But for the first time a non-transmissible set of determinants was observed. The Tc resistant transductants were still transmissible, but the Sm and Su resistant transductants could not be detected to transfer Sm or Su resistance at all. This was not a feature of a particular P1 lysate, or of a rare event, since a second P1 lysate was prepared and showed the same result, and over 20 colonies were tested on that occasion. Nor, it seemed, was it solely a feature of the strain J5-3 R313, since the Sm resistant transductants lack sex factor activity if the phage P1 lysate was prepared on the strain J5-3 R313-SS-1.

It was now concluded that the Tc resistance and hsII determinants were attached to a sex factor. Two possibilities were considered for the status of the Sm and Su resistance determinants: firstly that they were on a plasmid, and a second sex factor, carrying no detected determinants, was also present; secondly, that the Sm and Su resistance determinants are part of an R factor, but this R factor cannot be transduced ~~as an~~ in its entirety by phage P1. The idea that the phage P1 does not always transduce an entire R factor has also been suggested by Romero & Meynell (1969), but only to explain rare, otherwise anomalous, transductants.

In Salmonella, R factors are not transduced intact by the

phages P22, epsilon 15 or epsilon 34. Recombinants with the phage genome, and integration into the chromosome via integration of the phage are reported, and transductants from the epsilon phages have been shown to become transmissible again by recombination with an F factor (Harada et al., 1963; 1964; Dubnau & Stocker, 1964). It was therefore interesting to see if the transductants were transmissible when in the presence of another sex factor, and strains were constructed to test this. No transfer of drug resistance was observed when the sex factor was the fi$^+$ R factor, R124, nor when it was the segregant of R313, R313-T-1 (an fi$^-$ R factor), although both superinfecting R factors transferred normally. The F factor caused a low level of transfer of Sm and Su resistance determinants, the level being strikingly low when regarded as a percentage of cells capable of transferring F. Although transfer of F was not measured, it was assumed to be in the order of 50-100% under the conditions used, whereas only one donor in 10^{-6} was transferring resistance to Sm. In contrast, the fi$^-$ R factor, R143, transferred the Sm resistance at about 10^{-5}/donor cell, and as it was repressed, the Km resistance (attached to the sex factor) was only transferred at a frequency of 10^{-3}/donor cell. Hence the efficiency with which R143 mobilised the Sm resistance determinants was very much greater than that with which any other of the sex factors was found to cause mobilisation. F was not causing mobilisation by recombination to give a transmissible hybrid structure, because the ex-conjugants which are Sm resistant and are derepressed for F (plate M82) did not then transfer Sm resistance at a higher frequency than the original Sm resistant F$^+$ strain. Nor did colonies apparently F$^-$, but Sm resistant, after

transfer of Sm resistance by F, contain a sex factor, since they failed to transfer. Mobilisation of the Sm resistance by F could be due to F mediated transfer of chromosomal genes, or to chance transfer of a non-transmissible plasmid via the transfer apparatus of the F. But it is possible that the Sm resistance determinants are attached to a defective sex factor, whose missing functions are only poorly supplied by F, or an fi$^+$ R factor (R124), but are supplied very efficiently by the fi$^-$ R factor R143. If, as would appear at first sight, mobilisation is dependent on the presence of an fi$^-$ sex factor, it is surprising that R313-T-1, the fi$^-$ segregant of R313, does not supply the necessary functions. However, if two compatible fi$^-$ R factors had been carried in the original R313 strain, it is possible that one of these would fail to supply the necessary functions if a defect occurred in the transfer factor of the other.

Analysis of ex-conjugants showed for the strains originally carrying R143 and the Sm resistance determinant, precisely the type of pattern obtained for mobilisation of colE1 by colI - i.e. of a non-transmissible plasmid by a sex factor. Also, the fact that R143 can mobilise the determinants at a frequency as high as 1% of its own transfer frequency argues against a chromosomal location for the Sm resistance determinants, since mobilisation of chromosomal genes by sex factors other than F is generally very low, e.g. by colI (Ozeki, Howarth & Clowes, 1961).

It is now certain that the J5-3 R313 carried not just one R factor, but an R factor (with Tc resistance and hsII determinants) and a plasmid carrying determinants for resistance to Sm and Su. Whether the second plasmid was also a sex factor, or there was a second sex factor in the strain, is not clear. The former possibility,

that the Sm and Su resistance determinants are associated with a sex factor, forming a second <u>fi</u>⁻ R factor in the strain, seems the more probable, but the presence of the third plasmid, a sex factor not associated with any detected determinants, is not ruled out.

CHAPTER 5

ISOLATION AND PROPERTIES OF RESTRICTIONLESS MUTANTS OF RI AND RII

A. Introduction

The fine structure genetic analysis of the host specificity (hs) genes of the Escherichia coli chromosome has been severely limited, particularly due to the lack of selection procedures for restriction and modification phenotypes. Phenotypically, only two classes of hs mutant could be observed, and while the nature and frequency of these two classes of mutant suggested a minimum number of genes, confirmation was delayed until the development of a complementation system, which revealed a minimum of three genes.

It is even more difficult to obtain a genetic fine structure analysis for the R factor carried hs determinants. In conjugation, R factors appear to only transfer the entire R factor, so that time of entry studies, or mapping by linkage in genetic crosses, cannot be used to investigate the fine structure. Phage P1 generally transduces the whole R factor so that co-transduction with phage P1 cannot be used to map R factors effectively.

Hence the investigation of the genetic structure of the hs determinants carried by R factors is very much dependent on the information obtained by the study of mutant types, and from possible complementation studies.

B. Isolation of Restrictionless Mutants of RI and RII

i Isolation of spontaneous r^-m^+ mutants of RII

In the course of the experiments presented in chapter 3, two spontaneous r^-m^+ mutants of RII were isolated in J5-3 R^+ strains.

As stated in chapter 2 section E, it was customary to use only a single colony as the inoculum for an overnight culture in broth. Twice the single colony of a group II strain exhibited no restriction, and after purification of the new strain from the culture, was shown to have the phenotype r^-m^+II (i.e. phenotypically restrictionless, but retaining group II type modification). One mutant, R199-1, was derived from R199, the other, R313-1, from R313, and both are r^-m^+II with respect to all the phages normally restricted by hsII (table 2.5.).

Further attempts to obtain other spontaneous mutants from R313 by testing several hundred colonies for restriction, by the method in chap. 2 section G.ii.d, did not yield any other r^- mutants. Likewise no r^- mutants of R124 were found by screening unselected colonies for restriction. It was therefore necessary to use a selection procedure for the isolation of more r^- mutants.

ii Isolation of restrictionless mutants of RI and RII by EMS mutagenesis and a selection procedure

The selection procedure (chap. 2 section L) requires a strain lysogenic for the heat inducible prophage λ CI_{857}. The selection of r^- cells from the culture relies on lysogenisation of these cells with a wild type phage λ which is restricted by r^+ cells. The culture is induced by destruction of the repressor of phage λ CI_{857} at 42^o, but cells also lysogenised by wild type phage λ are not killed by this heat treatment, as the intact repressor of the wild type phage λ protects the culture from induction. Although restrictionless survivors ought to be lysogenic for wild type phage λ, and therefore to grow at 37^o, the yield of survivors at 37^o was lower by up to 100 fold compared to the yield at 30^o. This was not

accompanied by an enrichment for r^- mutants at 37°, so all incubations of strains after heat induction were at 30°. It is possible that the double lysogeny is unstable.

Three R factors were chosen for the isolation of r^- mutants: R124, the only RI; R313-T-1, which carried determinants for Tc resistance and hsII; and R132-SS-1, also carrying the hsII determinants, but determinants for resistance to Sm and Su. To make the strains required for the selection procedure, the R factors were transferred from J5-3 R^+ donors to a C600 λ CI_{857} recipient, using 30° for the mating instead of the normal 37° (chap. 2 section O.ii.) to avoid induction of the prophage. All subsequent incubations of the strains were at 30°. In the case of R313-T-1 and R132-SS-1, the original J5-3 R^+ strains were not used as donors since these were complex, and transfer of other resistance determinants might have occurred.

The EMS mutagenesis procedure is described in chapter 2 section K. The results of five experiments, using EMS mutagenesis followed by the selection procedure, are listed in table 5.1. The experiments with R124 yielded 48 r^- colonies out of only 90 tested, and the two phenotypes, r^-m^- and r^-m^+, were found in approximately equal number (totals 28 and 20 respectively) and both types were found in each of the two experiments. A much poorer yield of r^- mutants was obtained with the RII^+ strains, R313-T-1 yielding only two r^-m^+ mutants out of a total of 70 colonies tested, and R132-SS-1 giving eight r^- colonies out of the 58 tested. The only r^-m^- mutants of RII were mutants of R132-SS-1, five out of the eight colonies found to be r^- were r^-m^-.

The seven mutants retained for study after purification are

Table 5.1.

Results of five experiments in which restrictionless mutants of RI or RII were found

Parent R factor	No. of colonies tested	No. of r^-m^+	No. of r^-m^-
R124	40	13	14
R124	50	7	14
R313-T-1	40	1	0
R313-T-1	30	1	0
R132-SS-1	58	3	5

Table 5.2.

Origin, host specificity phenotype and drug resistances of restrictionless mutants of RI and RII produced by EMS mutagenesis

Parent R factor	Mutant R factor	Drug resistances	Host specificity phenotype*
R124	R124-1	Tc	r^-m^+ I
R124	R124-2	Tc	r^-m^- I
R124	R124-3	Tc	r^-m^+ I
R132-SS-1	R132-1	Sm Su	r^-m^+ II
R132-SS-1	R132-2	Sm Su	r^-m^- II
R313-T-1	R313-2	Tc	r^-m^+ II
R313-T-1	R313-3	Tc	r^-m^+ II

* tested with phage λ

listed in table 5.2. (see also table 2.5.). Since it is possible that colonies of the same phenotype isolated in one experiment may have all arisen from one mutant cell, only one mutant of each phenotype is retained from any one experiment. Although the mutants retained for further study were "good", $r^{-}m^{-}$ or $r^{-}m^{+}$, some r^{-} mutants were seen with an intermediate phenotype for modification, i.e. they were $m^{-}/^{+}$.

C. The Uniqueness of the Host Specificities of RI and RII

A necessary control for possible complementation experiments was the demonstration that the host specificities of RI and of RII, were different from any host specificity with which complementation was attempted. Tables 5.3. to 5.6. contain the information proving that the host specificities hsI and hsII are distinct from the host specificities determined by phage P1 and the Escherichia coli chromosome(only host specificity types K, B and 15 tested). Phage λ.KRI and λ.KRII was restricted by K(P1), and phage λ.K(P1) by KRI and KRII (table 5.3.), so P1 does not determine the same host specificity as either RI or RII. Phage λ.15 was restricted by CRI and CRII, and phage λ.CRI and λ.CRII by 15 (table 5.4) hence the host specificities determined by 15 and RI and RII are different. Phage λ.CRI (table 5.5.) and phage λ.CRII (table 5.6.) were both restricted by K and by B, and phage λ.K and λ.B were both restricted by CRI and CRII, so that it is clear that neither K nor B host specificity is the same as hsI or hsII.

There are some features of tables 5.5. and 5.6. which require comment. Although the e.o.p. is a measurement very much dependent on "environmental" factors, some e.o.p. are consistently higher or lower than expected, despite the precautions of use of one batch of media and the same incubator. The main anomalies are the low e.o.p.

Table 5.3.

E.o.p. of phage λ grown on the strains

K, KRI, KRII and K(P1)

Host strain	Phage: λ.K	λ.KRI	λ.KRII	λ.K(P1)
K	1.0	1.0	1.0	1.0
KRI	4×10^{-4}	1.0	1×10^{-3}	3×10^{-4}
KRII	2×10^{-2}	2×10^{-2}	1.0	5×10^{-2}
K(P1)	1×10^{-4}	1×10^{-4}	1×10^{-4}	1.0

K is C600, RI is R124, and RII is R313-T-1

Table 5.4.

E.o.p. of phage λ grown on the strains

15, 15RI, 15RII, C, CRI and CRII

Host strain	Phage: λ.15	λ.15RI	λ.15RII	λ.C	λ.CRI	λ.CRII
15	1.0	1.0	1.0	3×10^{-2}	5×10^{-2}	1×10^{-2}
15RI	1×10^{-2}	1.0	1×10^{-2}	1×10^{-3}	1×10^{-2}	1×10^{-4}
15RII	1×10^{-2}	1×10^{-2}	1.0	1×10^{-2}	1×10^{-2}	1×10^{-2}
C	1.0	1.0	1.0	1.0	1.0	1.0
CRI	1×10^{-3}	1.0	1×10^{-5}	5×10^{-4}	1.0	1×10^{-4}
CRII	1×10^{-2}	1×10^{-2}	1.0	1×10^{-2}	1×10^{-2}	1.0

RI is R124, RII is R313-T-1

Table 5.5.

E.o.p. of phage λ grown on K, B, C, KRI, BRI and CRI

Host strain	Phage: λ.K	λ.KRI	λ.B	λ.BRI	λ.C	λ.CRI
K	1.0	1.0	$5x10^{-4}$	$1x10^{-3}$	$4x10^{-4}$	$1x10^{-4}$
KRI	$4x10^{-4}$	1.0	$7x10^{-5}$	$5x10^{-3}$	$7x10^{-5}$	$6x10^{-4}$
KRII	$2x10^{-2}$	$2x10^{-2}$	$7x10^{-5}$	$1x10^{-4}$	$2x10^{-4}$	$1x10^{-4}$
B	$2x10^{-4}$	$5x10^{-5}$	1.0	1.0	$1x10^{-4}$	$5x10^{-4}$
BRI	$2x10^{-4}$	$1x10^{-3}$	$7x10^{-5}$	1.0	$1x10^{-5}$	$2x10^{-4}$
BRII	$7x10^{-4}$	$6x10^{-3}$	$2x10^{-3}$	$6x10^{-3}$	$1x10^{-2}$	$7x10^{-4}$
C	1.0	1.0	1.0	1.0	1.0	1.0
CRI	$5x10^{-6}$	1.0	$7x10^{-6}$	1.0	$2x10^{-5}$	1.0
CRII	$4x10^{-3}$	$1x10^{-2}$	$1x10^{-4}$	$1x10^{-4}$	$1x10^{-2}$	$1x10^{-2}$

K is C600, B is Btrp⁻, C is Cthr⁻, RI is R124, RII is R313-T-1

Table 5.6.

E.o.p. of phage λ grown on K, B, C, KRII, BRII, and CRII

Host strain	Phage: λ.K	λ.KRII	λ.B	λ.BRII	λ.C	λ.CRII
K	1.0	1.0	$5x10^{-4}$	$5x10^{-4}$	$4x10^{-4}$	$1x10^{-4}$
KRI	$4x10^{-4}$	$1x10^{-3}$	$7x10^{-5}$	$5x10^{-5}$	$4x10^{-4}$	$2x10^{-5}$
KRII	$2x10^{-2}$	1.0	$7x10^{-5}$	$7x10^{-3}$	$2x10^{-4}$	$1x10^{-3}$
B	$2x10^{-4}$	$5x10^{-5}$	1.0	1.0	$1x10^{-4}$	$2x10^{-4}$
BRI	$2x10^{-4}$	$7x10^{-5}$	$7x10^{-5}$	$7x10^{-4}$	$1x10^{-5}$	$1x10^{-4}$
BRII	$7x10^{-4}$	$1x10^{-2}$	$2x10^{-3}$	1.0	$1x10^{-2}$	$7x10^{-2}$
C	1.0	1.0	1.0	1.0	1.0	1.0
CRI	$5x10^{-6}$	$1x10^{-4}$	$7x10^{-6}$	$2x10^{-5}$	$2x10^{-5}$	$5x10^{-5}$
CRII	$4x10^{-3}$	1.0	$1x10^{-4}$	1.0	$1x10^{-2}$	1.0

K is C600, B is B trp⁻, C is C thr⁻, RI is R124, RII is R313-T-1

of some phages on CRI and CRII, and the high e.o.p. of some phages on BRII. Phages λ.K and λ.B have e.o.p. on CRI and CRII in the order of ten fold greater than those obtained when the same phages were plated on KR$^+$ or BR$^+$ strains. When testing R$^+$ strains which were restrictionless mutants of K or B, the abnormally low e.o.p. of phages λ.K and λ.B on these strains was also noted. The high e.o.p. on BRII is particularly noticeable for phages λ.C, λ.KRI and λ.KRII. Since the strain has not been cured, it cannot be ruled out that the RII is efficiently selecting restrictionless mutants of B, though independent BRII strains have been made and all shown to have identical behaviour. If selection of mutants is occurring they are r$^-$m$^+$, since these strains show m$^+$B phenotype. A high e.o.p. of phages λ.15 and λ.15RII on 15RI is similarly noticeable in table 5.4., though this has not been investigated.

D. Absence of Complementation

For the complementation tests, the presence of a particular host specificity was based on the restriction of phage λ modified for the other host specificities carried by the strain, and modification of the phage λ for each host specificity was checked separately. For example, when testing the strain BRI, the presence of both B and RI host specificities is detected by the restriction of both phages λ.B and λ.CRI, and the equal plating of phage λ.BRI on strains B, C, CRI and BRI.

All the restrictionless mutants of RI and RII were tested for complementation with the intact host specificity genes of K and B (table 5.7.), and the mutants of RI and three r$^-$m$^+$ mutants of RII were tested with phage P1 host specificity for complementation (table 5.8.). No restoration of restriction for r$^-$m$^+$ mutants, or

Table 5.7.

Restriction and modification phenotypes of restrictionless mutants of R124, R132, and R313-T1 in r^+m^+K and r^+m^+B

R factor	Host specificity* phenotype in: r^+m^+K		r^+m^+B	
R124	r^+m^+K	r^+m^+I	r^+m^+B	r^+m^+I
R124-1	r^+m^+K	r^-m^+I	r^+m^+B	r^-m^+I
R124-2	r^+m^+K	r^-m^-I	r^+m^+B	r^-m^-I
R124-3	r^+m^+K	r^-m^+I	r^+m^+B	r^-m^+I
R313-T-1	r^+m^+K	r^+m^+II	r^+m^+B	r^+m^+II
R313-1	r^+m^+K	r^-m^+II	r^+m^+B	r^-m^+II
R313-2	r^+m^+K	r^-m^+II	r^+m^+B	r^-m^+II
R313-3	r^+m^+K	r^-m^+II	r^+m^+B	r^-m^+II
R199-1	r^+m^+K	r^-m^+II	r^+m^+B	r^-m^+II
R132-1	r^+m^+K	r^-m^+II	r^+m^+B	r^-m^+II
R132-2	r^+m^+K	r^-m^-II	r^+m^+B	r^-m^-II

The phenotypes are tested in two r^+m^+K strains, C600 and J5-3. The r^+m^+B strain is B try^-,

* tested using phage λ

Table 5.8.

Host specificity phenotypes of C600(P1) R* strains

R factor	Host specificity phenotype*		
R124	r^+m^+K	r^+m^+P1	r^+m^+I
R124-1	r^+m^+K	r^+m^+P1	r^-m^+I
R124-2	r^+m^+K	r^+m^+P1	r^-m^-I
R124-3	r^+m^+K	r^+m^+P1	r^-m^+I
R313-T-1	r^+m^+K	r^+m^+P1	r^+m^+II
R313-2	r^+m^+K	r^+m^+P1	r^-m^+II
R313-3	r^+m^+K	r^+m^+P1	r^-m^+II
R199-1	r^+m^+K	r^+m^+P1	r^-m^+II

* tested with phage λ

Table 5.9.

The phenotypes of C600 strains carrying R132-SS-1 and R124 or an R124 mutant

Strain	Host specificity phenotype*		
K RII R124	r^+m^+K	r^+m^+II	r^+m^+I
K RII R124-1	r^+m^+K	r^+m^+II	r^-m^+I
K RII R124-2	r^+m^+K	r^+m^+II	r^-m^-I
K RII R124-3	r^+m^+K	r^+m^+II	r^-m^+I

K is C600, RII is R132-SS-1

* tested using phage λ

Table 5.10.

The host specificity phenotypes of restrictionless mutants of K and B carrying RI and RII

Phenotype of host strain	R factor: RI(r^+m^+I)	RII(r^+m^+II)
r^+m^+K	r^+m^+K r^+m^+I	r^+m^+K r^+m^+II
r^-m^+K	r^-m^+K r^+m^+I	r^-m^+K r^+m^+II
r^-m^-K	r^-m^-K r^+m^+I	r^-m^-K r^+m^+II
r^+m^+B	r^+m^+B r^+m^+I	r^+m^+B r^+m^+II
r^-m^+B	r^-m^+B r^+m^+I	r^-m^+B r^+m^+II
r^-m^-B	r^-m^-B r^+m^+I	r^-m^-B r^+m^+II

The r^-m^+K is 7K, the r^-m^-K is 4K, the r^-m^+B is B6, the r^-m^-B is B8, RI is R124, RII is R313-T-1.
Host specificity phenotype tested using phage λ.

restriction and modification of r^-m^- mutants was observed, although the RI and RII host specificities are expressed in K and B (lines one and five, table 5.7) and P1 and RI (or RII) host specificities are both expressed in K (lines one and five, table 5.8.). The RI mutants were also tested with an intact RII (R132-SS-1) host specificity and no complementation observed, although RI and RII host specificities are operational in the same cell (table 5.9.).

Mutants of B and K (r^-m^- and r^-m^+) were also tested for complementation with intact hsI and hsII determinants. Some importance was attached to these results as the restrictionless mutants of RI and RII were obtained in K, and hence are pre-selected for lack of complementation with K strains. Again no restoration of restriction to r^-m^+ mutants, or restriction and modification to r^-m^- mutants was observed (table 5.10.).

E. Discussion

No fine structure genetic analysis of the host specificity determinants of the Escherichia coli chromosome has so far been possible, mainly due to the lack of a true selective procedure for selecting for either of the phenotypic characters of restriction and modification. However, some idea of the number of genes involved can be gained from the phenotypes and frequencies of the mutants found. It is possible to select for restrictionless mutants, though the selection techniques, such as use of restricted phage λdg (Wood, 1965), Flac (Glover & Colson, 1966) and R factors (Arber & Morse, 1964) are not absolute. Modification cannot be selected, though colonies can be screened for modification by a replica plating method; however, no mutant with the r^+m^- phenotype has been found. The restrictionless (r^-) mutants found fall into two classes which are encountered in approximately equal numbers, often in a single experiment. These two types are the r^-m^- and the r^-m^+ mutants, and since the r^-m^- is as frequent as the r^-m^+, it is assumed to result from a single mutation. It was suggested on these data that the host specificity of E. coli strains K and B was controlled by a minimum of three genes, and this minimum of three genes has been confirmed by complementation studies (Arber, 1969; Boyer & Roulland-Dussoik, 1969; S. W. Glover, personal communication). These three genes are assumed to be contiguous on the E. coli chromosome as they are all cotransducible with serB by phage P1.

The three genes have provisionally been termed hss, hsr and hsm (chap. 1 section E.i.). The model which has been suggested is that the hss gene determines a protein sub unit which is common to the restriction and the modification enzymes. A mutation in this gene

produces the r^-m^- phenotype in a single mutational step; it is suggested, and the complementation data support the suggestion, that this gene controls the specificity of the enzymes. The hsr gene is that in which a mutation produces the r^-m^+ phenotype; it is assumed that the gene codes for a protein sub unit required by the restriction enzyme only. The hsm gene is assumed to control production of a sub unit required by the modification enzyme only, and mutations in this gene are found only in association with mutations in hsr gene. The possibility that the restriction enzyme is in fact an oligomer is supported by observations of Meselson & Yuan (1968).

This is the simplest model to explain the data. Mutants of phenotype intermediate between r^+m^+ and r^-m^- have been suggested as possible regulatory mutants by Boyer & Roulland-Dussoix (1969), who also observed a mutation of a type not previously encountered - the trans-dominant r^-. This evidence has been used to suggest that there may be a fourth gene involved in host specificity.

The host specificity genes of K and B are allelic, and it was not surprising that mutants of the two strains will complement each other. It is surprising that the mutants of the third allelic chromosomal host specificity, 15, were not also found to complement with the K and B mutants (Arber, 1969). The host specificity carried by the phage P1 also had not been found to complement with the chromosomal genes.

i The uniqueness of the host specificities of RI and RII

In the course of necessary control experiments as preliminaries to complementation studies, it became clear that the host

specificities carried by RI and RII were different from those of Escherichia coli K, B and 15, and from the phage P1 host specificity. The main interest in this information was in its possible bearing on the origin of the R factor associated genes. It is possible that R factors carry various genetic determinants as a result of exchanges with bacterial or phage chromosomes. If this is so, and the host specificities have not altered since the exchange, then the hsI and hsII genetic determinants were not acquired from either the E. coli strains K, B or 15, or from the phage P1. As at least one other E. coli strain, strain W, is known to have a different host specificity (Kerszman, Glover & Aronovitch, 1967) from K and B, and other temperate phages carry host specificity determinants, e.g. a Salmonella phage (Anderson, 1955) it would have been good fortune if the origin of the hsI or hsII genes had been found easily.

ii Anomalous e.o.p. of phage λ

When P1 is present in a K strain, the restriction of phage λ.C is increased, though the e.o.p. does not fall in a simple fashion when P1 is introduced into K (phage λ.C plates with an e.o.p. of about 10^{-4} on K, 10^{-4} on C(P1) and 10^{-6} on K(P1)). There is no significant decrease in e.o.p. of phage λ.C on KRI or KRII compared with the e.o.p. observed on K. However, some anomalous e.o.p. of phage λ were observed. It appears that restrictionless strains (C or mutants of K and B) allow greater restriction (as measured by the e.o.p.) of phage λ by the hsI and hsII determinants, while B allows less restriction by these determinants. In the case of the B strains, little can be said, as they have not been cured and proved to be still r^+B, but it is interesting to note that Arber & Morse (1964) had observed an anomalous plating of phage λ on a BR^+ strain (the R factor carried is now known to be an RII type), but in contradiction

to the results here, the e.o.p. of phage λ.B on BR^+ was much lower than that of phage λ.C on CR^+. As little is known about the causes of physiological changes in restriction levels, nothing can be concluded from these observations, except that the interaction of host specificities may be very complex!

iii The restrictionless mutants of RI and RII

The first two mutants isolated were spontaneous mutants observed during the early testing of the R^+ strains. Both were of the r^-m^+ phenotype, and since the r^- phenotype of a mutant of RII can be expressed in the presence of the intact K host specificity genes, it is implied that the hsII determinants do not complement with the K host specificity. A criticism has recently been levelled at this type of deduction when applied to host specificity mutants of phage P1. Boyer & Roulland-Dussoix (1969) have pointed out that by selecting the mutants in a K strain there is a selection for mutants which will not complement with the K host specificity. Such mutants are also unlikely to complement with host specificity determinants which complement with K. However, since these were spontaneous mutants, and no selection pressure had been applied, it is improbable that a class of r^-m^+ mutant also exists which will complement with K, particularly as the intact hsI and hsII determinants will not complement mutants of K or B, see section E.iv. of this chapter.

The existence of r^-m^+ mutants suggested that the hsI was analogous with the K and B host specificities in possessing minimum of three genes. The other r^- mutants of both RI and RII were also isolated in K strains, as observations mentioned in the preceding paragraph were taken to indicate that hsII and hsI do not complement with the K chromosome. Restrictionless mutants of two types, r^-m^+

and r^-m^-, have been isolated for both of the R factor carried host specificities, though only one experiment with an RII yielded any r^-m^- mutants. The two simplest models for the hsI and hsII determinants involve either two genes or three genes, and as the latter would be in line with the model for the K and B host specificities, having hss, hsr and hsm genes, it is preferred. But it is not possible on this data to exclude the alternative model, that there are two genes, hsr and hsm, and the restriction enzyme, product of hsr, also requires the product of the hsm gene to function.

iv The absence of complementation

To distinguish between alternative models for the genetic structure of the hsI and hsII determinants, it was hoped that complementation between mutants of RI and RII and some other system would be observed. Since all the R factors of group II are fi^-, and there is only one group I R factor, intra-group complementation was prevented, forced maintenance of two mutants of group II, i.e. two fi^- R factors in the same cell, would be likely to produce recombinants rather than set up a stable coexistence of the two R factors.

It was decided that the minimal test of complementation was the restoration of restriction of its own specificity to an r^-m^+ mutant by combining it with an intact set of different host specificity determinants. This requires interaction of the hss gene of one host specificity with the hsr gene of another (interaction being assumed to be at the protein sub unit stage). No such complementation was ever observed between mutants of RI and RII, P1, K, or B or between mutants of RII and P1, K or B. Nor were r^-m^+ (or r^-m^-) mutants of K and B complemented by the presence of intact

RI or RII host specificities. Hence no further data concerning the genetic structure of the hsI or hsII determinants is available.

It is still suggested that the host specificities of RI and RII, though distinct from other known host specificities, and failing to complement with any other host specificity determinants, can still be explained on the same genetic model as is used for the K and B host specificities. This implies the existence of a minimum of three genes involved in determining each host specificity, and so far the only available evidence is the existence of two phenotypes of restrictionless mutants.

Abbreviations

The standard abbreviations used are generally as recommended by the Biochemical Journal Suggestions and Instructions to Authors.

The abbreviations for genetic markers are those recommended by Demerec, Adelberg, Clark & Hartman (1966), with the following exceptions:-

ile	isoleucine requirement
hs	host specificity, all the genes involved in. The type of the specificity is indicated by the following letter, e.g. hsK represents all the genes required for K host specificity.
hss	gene in which mutation causes the host specificity phenotype r^-m^-
hsr	gene in which a mutation causes the host specificity phenotype r^-m^+
hsm	gene in which a mutation causes the m^- phenotype, in the presence of a mutation in hsr
λ^R	resistance to phage λ
$colI^R$	resistance to colicin I

The following are other abbreviations used in this thesis:

I, II used as suffixes to denote host specificity or an R factor carrying the host specificity, characteristic of groups I and II respectively, as:-

RI R factor carrying hsI

RII R factor carrying hsII

r restriction phenotype, presence or absence indicated in superscript by $^+$ or $^-$

m modification phenotype, presence or absence indicated in superscript by $^+$ or $^-$

Tc tetracycline

Sm streptomycin

Su sulphonamide

Km kanamycin

Cm chloramphenicol

Acknowledgements

Limitations of space prevent me from thanking individually all who have helped, directly and indirectly, in the preparation of this thesis, but I have two particular debts to acknowledge. The first is to Dr. S. W. Glover, for all his help during the last three years. The second is to Drs. N. Datta and E. Meynell, for without their generous gift of R^+ strains, this investigation could not have been commenced.

I am also indebted to the MRC for financial support (Scholarship for Training in Research Methods, G.77/1298).

References

Adams, M. H. (1950) Methods for the study of bacterial viruses. Methods in Medical Research, 2: 1.

Adelberg, E. A. and Burns, S. N. (1960) Genetic variation in the sex factor of Escherichia coli. J. Bact., 79: 321-330.

Anderson, E. S. (1955) Consideration of the Vi-phage types of Salmonella typhi on a structural basis. Nature, 175: 171.

Anderson, E. S. (1966) Influence of the Transfer factor on the phage sensitivity of Salmonella. Nature, 212: 795-799.

Anderson, E. S and Datta, N. (1965) Resistance to penicillins and its transfer in Enterobacteriaceae. Lancdt 1: 407-409.

Anderson, E. S. and Felix, A. (1952) Variation in Vi-phage II of Salmonella typhi. Nature, 170: 492-493.

Anderson, E. S. and Lewis, M. J. (1965) Characterisation of a transfer factor associated with drug resistance in Salmonella typhimurium. Nature, 208: 843-849.

Appleyard, R. K. (1954) Segregation of new lysogenic types during growth of a doubly lysogenic strain derived from Escherichia coli K12. Genetics, 39: 440-452.

Arber, W. (1965) Host specificity of DNA produced by Escherichia coli. V. The role of methionine in the production of host specificity. J. Mol. Biol., 11: 247-256.

Arber, W. (1966) Host specificity of DNA produced by Escherichia coli. 9. Host controlled modification of bacteriophage fd. J. Mol. Biol., 20: 483-496.

Arber, W. (1968) Host-controlled Restriction and modification of bacteriophage. Symposium Soc. Gen. Microbiol. XVIII. The Molecular Biology of Viruses. 295-314.

Arber, W. (1969) Ann. Rev. Biochem. In press.

Arber, W. and Dussoix, D. (1962) Host specificity of DNA produced by Escherichia coli. I. Host controlled modification of bacteriophage λ. J. Mol. Biol., 5: 18-36.

Arber, W., Hattman, S. and Dussoix, D. (1963) On the host controlled modification of bacteriophage λ. Virology, 21: 30-35.

Arber, W. and Lataste-Dorolle, C. (1961) Erweiterung des wirtsbereiches des bakteriophagen λ auf Escherichia coli B. Pathol. Microbiol., 24: 1012-1018.

Arber, W. and Morse, M. L. (1965) Host specificity of DNA produced by Escherichia coli. Effects on bacterial conjugation. Genetics, 51: 137-148.

Benzinger, R, (1968) Restriction of infectious bacteriophage fd DNA's and an assay for in vitro host-controlled restriction and modification. Proc. natn. Acad. Sci., U.S.A., 59: 1294-1299.

Bertani, G. (1951) Studies on lysogenesis. I. The mode of phage liberation by lysogenic Escherichia coli. J. Bact., 62: 293-300.

Bertani, G. and Weigle, J. J. (1953) Host controlled variation in bacterial viruses. J. Bact., 65: 113-121.

Boice, L. B. and Luria, S. E. (1963) Behaviour of prophage P1 in bacterial matings. I. Transfer of the defective prophage P1 dl. Virology, 20: 147-157.

Bouanchaud, D. H., Scavizzi, M. R. and Chabbert, Y. A. (1969) Elimination by ethidium bromide of antibiotic resistance in Enterobacteria and Staphylococci. J. gen. Microbiol., 54: 417-425.

Boyer, H. (1964) Genetic control of restriction and modification in Escherichia coli. J. Bact., 88: 1652-1660.

Boyer, H. W. and Roulland-Dussoix, D. (1969) A complementation analysis of the restriction and modification of DNA in Escherichia coli. J. Mol. Biol., 41: 459-472.

Boyle, J. M., Ritchie, D. A. and Symonds, N. (1965) Density gradient separation of host-modified bacteriophage T2. J. Mol. Biol. 14: 48-54.

Brinton, C. C., Gemski, P. and Carnahan, J. (1964) A new type of bacterial pilus genetically controlled by the fertility factor of E. coli K12 and its role in chromosome transfer. Proc. natn. Acad. Sci., U.S.A., 52: 776-783.

Caro, L. G. and Schnös, M. (1966) The attachment of the male-specificic bacteriophage F1 to sensitive strains of Escherichia coli. Proc. natn. Acad. Sci., U.S.A., 56: 126-132.

Christensen, J. R. (1964) Further studies of host-controlled modification of bacteriophages P2 and T1. Virology, 24: 270-277.

Clowes, R. C. (1965) Colicin factors as sex factors. Symp. Biol. Hung., 6: 85-95.

Clowes, R. C. and Rowley, D. (1954) Some observations on linkage effects in genetic recombination in Escherichia coli K12. J. Gen. Microbiol., 11: 250-260.

Colson, C. and Colson, A. M. (1967) Host specificity and fertility in Salmonella typhimurium LT7. B.B. Res. Comm., 29: 692-695.

Colson, C., Glover, S. W., Symonds, N. and Stacey, K. A. (1965) The location of the genes for host-controlled modification and restriction in Escherichia coli K12. Genetics, 52: 1043-1050.

Crawford, E. M. and Gesteland, R. F. (1964) The adsorption of bacteriophage R-17. Virology, 22: 165-167.

Datta, N. and Kontamichalou, P. (1965) Penicillinase synthesis controlled by infectious R factors in Enterobacteriaceae. Nature, 208: 239-241.

Datta, N. and Richmond, M. H. (1966) The purification and properties of a penicillinase whose synthesis is mediated by an R factor in Escherichia coli. Biochem. J., 98: 204-209.

Davis, J. E., Strauss, J. H. and Sinsheimer, R. L. (1961) Bacteriophage MS2: another RNA phage. Science, N.Y., 134: 1427.

De Haan, P. G., Stouthamer, A. H., Felix, H. S. and Mol, A. K. (1963) Transfer of F' from Escherichia coli K12 to Escherichia coli B and to strains of Paracolobacter and Klebsiella. Antonie van Leeuwenhoek, 29: 407-420.

Demerec, M., Adelberg, E. A., Clark, A. J. and Hartman, P. (1966) A proposal for a uniform nomenclature in Bacterial Genetics. Genetics, 54: 61-76.

Dettori, R., Maccacaro, G. A. and Piccinin, G. L. (1961) Sex-specific bacteriophages of Escherichia coli K12. Giornale da Microbiologia, 9: 141-150.

Dubnau, E. and Stocker, B. A. D. (1964) Genetics of plasmids in Salmonella typhimurium. Nature, 204: 1112-1113.

Dunn, D. B. and Smith, J. D. (1958) The occurrence of 6-Methyl aminopurine in Deoxyribonucleic acids. Biochemical Journal, 68: 627.

Dussoix, D. and Arber, W. (1962) Host specificity of DNA produced by Escherichia coli. II. Control over acceptance of DNA from infecting phage λ. J. Mol. Biol., 5: 37-49.

Dussoix, D. and Arber, W. (1965) Host specificity of DNA produced by Escherichia coli. IV. Host specificity of infections DNA from bacteriophage lambda. J. Mol. Biol., 11: 238-246.

Egawa, R., Sawai, T. and Mitsuhashi, S. (1967) Drug resistances of enteric bacteria. XII. Unique substrate specificity of penicillinase produced by R factor. Japan J. Microbiol., 11: 173-178.

Erikson, R. L. and Szybalski, W. (1964) The Cs_2 SO_4 equilibrium density gradient and its application for the study of T-even phage DNA: glucosylation and replication. Virology, 22: 111-124.

Eskridge, R. W., Weinfeld, H. and Paigen, K. (1967) Susceptibility of different coliphage genomes to host-controlled variation. J. Bact., 93: 835-844.

Falkow, S., Citarella, R. V., Wohlheiter, J. A. and Watanabe, T. (1966) The molecular nature of R-factors. J. Mol. Biol., 17: 102-116.

Fredericq, P. (1946) Sur la pluralité des récepteurs d'antibiose de E. coli. C.R. Seance. Soc. Biol., 140: 1189-1191.

Franklin, N. & Dove (1969) In press.

Fukasawa, T. (1964a) The course of infection with abnormal bacteriophage T4 containing non-glucosylated DNA on Escherichia coli strains. J. Mol. Biol., 9: 525-536.

Fukasawa, T. (1964b) Localisation of restricting function of E. coli strains to host-modified T-even phages. Fed. Proc., 23: 272.

Fukasawa, T. and Saito, S. (1963) Abnormal growth of T-even phages in bacterial mutants defective in UDPG Pyrophosphorylase. Fed. Proc., 22: 406.

Fukasawa, T. and Saito, S. (1964) The course of infection with T-even phages on mutants of Escherichia coli K12 defective in the synthesis of uridine diphosphoglucose. J. Mol. Biol., 8: 175-183.

Gefter, M., Hausmann, R., Gold, M. and Hurwitz, J. (1966) The enzymic methylation of ribonucleic acid and deoxyribonucleic acid. X. Bacteriophage T3-induced S-adenosylmethionine cleavage. J. Biol. Chem., 241: 1995-2005.

Glover, S. W. and Colson, C. (1966) Stable and unstable alterations of the host-induced modification properties of Escherichia coli B, K and C. Genet. Res., 7: 223-234.

Glover, S. W. and Colson, C. (1969) Genetics of host-controlled restriction and modification in Escherichia coli. Genet. Res., Camb., 13: 227-240.

Glover, S. W., Schell, J., Symonds, N. and Stacey, K. A. (1963) The control of host-induced modification by phage P1. Genet. Res., 4: 480-482.

Gold, M. and Hurwitz, J. (1963a) Enzymic methylation of nucleic acids. Fed. Proc., 22: 230.

Gold, M. and Hurwitz, J. (1963b) The enzymic methylation of the nucleic acids. Cold Spring Harb. Symp. quant. Biol., 28: 140-156.

Gold, M., Hausmann, R., Maitra, U. and Hurwitz, J. (1964) The enzymatic methylation of RNA and DNA, VIII. Effects of bacteriophage infection on the activity of the methylating enzymes. Proc. natn. Acad. Sci., U.S.A., 52: 292-297.

Gough, M. and Lederberg, S. (1966) Methylated bases in the host-modified deoxyribonucleic acid of Escherichia coli and bacteriophage λ. J. Bact., 91: 1460-1468.

Guinee, P. A. M. & Willems, H. M. C. C. (1967) Restriction and modification of phage 47 and lambda by R factors. Antonie van Leeuwenhoek, 33: 397-406.

Harada, K., Kameda, M., Suzuki, M., Kakinuma, Y. and Mitsuhashi, S. (1961) Drug resistance of enteric bacteria. 13. Interference of R factors in the R^t bacterial cell when infected with another R factor. Gunma. J. med. Sci., X: 201-205.

Harada, K., Kameda, M., Suzuki, M. and Mitsuhashi, S. (1963) Drug resistance of enteric bacteria. II. Transduction of transmissible drug-resistance (R) factors with phage epsilon. J. Bact., 86: 1332-1338.

Harada, K., Kameda, M., Suzuki, M. and Mitsuhashi, S. (1964) Drug resistance of enteric bacteria. III. Acquisition of transferability of non-transmissible R(TC) factor in co-operation with F factor and formation of FR(TC). J. Bact., 88: 1257-1265.

Hattman, S. (1964) The functioning of T-even phages with unglucosylated DNA in restricting Escherichia coli host cells. Virology, 24: 333-348.

Hattman, S. and Fukasawa, T. (1963) Host-induced modification of T-even phages due to defective glucosylation of their DNA. Proc. natn. Acad. Sci., U.S.A., 50: 297-300.

Hattman, S., Revel, H. R. and Luria, S. E. (1966) Enzyme synthesis directed by non-glucosylated T-even Bacteriophages in restrictive hosts. Virology, 30: 427-438.

Hausmann, R. and Gold, M. (1966) Enzymic methylation of ribonucleic acid and deoxyribonucleic acid. IX. Deoxyribonucleic acid methylase in bacteriophage infected Escherichia coli. J. Biol. Chem., 241: 1985-1994.

Hirota, Y. (1960) The effect of acridine dyes on mating type factors in Escherichia coli. Proc. natn. Acad. Sci., U.S.A. 46: 57-64.

Hirota, Y., Nishimura, Y., Ørskov, F. and Ørskov, I. (1964) Effect of drug resistance factor R on the F properties of Escherichia coli. J. Bact., 87: 341-351.

Hirsch-Kauffman, M. and Sauerbier, W. (1968) Inhibition of modification and restriction for phages λ and T1 by co-infecting T3. Molec. Gen. Genetics, 102: 89-94.

Hoekstra, W. P. M. and De Haan, P. G. (1965) The location of the restriction locus for λ.K in Escherichia coli B. Mutation Research, 2: 204-212.

Hoffmann-Berling, H. &Mazé, R. (1964) Release of male-specific bacteriophages from surviving host bacteria. Virology, 22: 305-313.

Ihler, G. and M. Meselson (1963) Genetic recombination in bacteriophage λ by breakage and joining of DNA molecules. Virology, 21: 7-10.

Jacob, F. and Wollman, E. L. (1954) Etude génétique d'un bactériophage tempéré d'Escherichia coli: I. Le systeme génétique du bactériophage λ. Ann. Inst. Pasteur, 87: 653-673.

Kaiser, A. D. (1962) The production of phage chromosome fragments and their capacity for genetic transfer. J. Mol. Biol., 4: 275-287.

Kaiser, A. D. and Hogness, D. S. (1960) The transformation of Escherichia coli with deoxyribonucleic acid isolated from bacteriophage λ. J. Mol. Biol., 2: 392-415.

Kellenberger, G., Symonds, N. and Arber, W. (1966) Host specificity of DNA produced by Escherichia coli. 8. Its acquisition by phage λ and its persistence through consecutive growth cycles. Z. Vererbungsl., 98: 247-256.

Keller, E. F. (1964) Single burst analysis for semiconserved phage. Virology, 22: 649-650.

Kerszman, G., Glover, S. W. & Aronovitch, J. (1967) The restriction of bacteriophage λ in Escherichia coli strain W. J. Gen. Virol., 1: 333-347.

Kitamoto, O., Kasai, K., and Fukaya, A. (1956) Drug sensitivity of the Shigella strains isolated in 1955. J. Japan Assoc. Infectious Diseases, 30: 403-404.

Klein, A. (1965) Mechanismen der wirtskontrollierten modifikation des phagen T1. Z. Vererbungsl., 96: 346-363.

Klein, A. and Sauerbier, W. (1965) Role of methylation in host controlled modification of phage T1. B.B. Res. Comm., 18: 440-445.

Kondo, E., Harada, K. and Mitsuhashi, S. (1962) Drug resistance of enteric bacteria. 12. Transduction of the transmissible drug resistance factor by bacteriophage P1kc. Jap. J. exp. Med., 32: 139-147.

Kornberg, A., Zimmerman, S. B., Kornberg, S. R. and Josse, J. (1959) Enzymatic synthesis of deoxyribonucleic acid. VI. Influence of bacteriophage T2 on the synthetic pathway in host cells. Proc. natn. Acad. Sci., U.S.A., 45: 772-785.

Lawn, A. M., Meynell, E., Meynell, G. G. and Datta, N. (1967) Sex pili and the classification of sex factors in the Enterobacteriaceae. Nature, 216: 343-346.

Lebek, G. (1963) Uber die enstehung mehrfachresistanter Salmonellen. Ein experimenteller beitrag. Zentr. Bakteriol. Parasitenk. abt. I. Orig., 188: 494-505.

Lebek, G. (1969) Die infectiose bakterielle antibiotika-resistenz.

Lederberg, S. (1957) Suppression of the multiplication of heterologous bacteriophages in lysogenic bacteria. Virology, 3: 496-513.

Lederberg, S. (1966) Genetics of host controlled restriction and modification of deoxyribonucleic acid in Escherichia coli. J. Bact., 91: 1029-1036.

Lederberg, J., Cavalli, L. L. and Lederberg, E. M. (1952) Sex compatibility in Escherichia coli. Genetics, 37: 720-730.

Lederberg, E. M. and Lederberg, J. (1953) Genetic studies of lysogenicity in Escherichia coli. Genetics, 38: 51-64.

Ledinko, N. (1964) Occurrence of 5-methylcytidylate in the DNA of phage lambda. J. Mol. Biol., 9: 834.

Linn, S. and Arber, W. (1968) Host specificity of DNA produced by Escherichia coli. X. In vitro restriction of phage fd replicative form. Proc. natn. Acad. Sci., U.S.A., 59: 1300-1306.

Luria, S. E. and Delbrück, M. (1943) Mutations of bacteria from virus sensitivity to virus resistance. Genetics, 28: 491-511.

Luria, S. E. and Human, M. L. (1952) A non-hereditary host-induced variation of bacterial viruses. J. Bact., 64: 557-569.

Matsushiro, A. (1961) Isolation of UV-inducible temperate phage ø80. Bikens J., 4: 133-135.

Meselson, M. and Yuan, R. (1968) DNA restriction enzyme from E. coli. Nature, 217: 1110-1114.

Meynell, E. and Datta, N. (1965) Functional homology of the sex-factor and resistance transfer factors. Nature, 207: 884-885.

Meynell, E. and Datta, N. (1966) The relation of resistance transfer factors to the F factor (sex factor) of Escherichia coli K12. Genet. Res., 7: 134-140.

Meynell, E. and Datta, N. (1967) Mutant drug resistance factors of high transmissibility. Nature, 214: 885-887.

Meynell, G. G. and Lawn, A. M. (1968) Filamentous phages specific for the I sex factor. Nature, 217: 1184-1186.

Meynell, E., Meynell, G. G. and Datta, N. (1968) Phylogenetic relationships of drug-resistance factors and other transmissible plasmids. Bacteriological Reviews, 32: 55-83.

Mitsuhashi, S., Harada, K. and Hashimoto, H. (1960a) Multiple resistance of enteric bacteria and transmission of drug-resistance to other strain by mixed cultivation. Jap. J. exp. Med., 30: 179-184.

Mitsuhashi, S., Harada, K., Kameda, M., Suzuki, M. and Egawa, R. (1960b) On the drug-resistance of enteric bacteria. 3. Transmission of the drug-resistance from Shigella to F⁻ on Hfr strains of E. coli K12. Jap. J. exp. Med., 30: 301-306.

Mitsuhashi, S., Harada, K., Kameda, M. (1961a) On the drug-resistance of enteric bacteria. 6) Spontaneous and artificial elimination of transmissible drug-resistance factors. Jap. J. exp. Med., 31: 119-123.

Mitsuhashi, S., Harada, K. and Kameda, M. (1961b) Elimination of transmissible drug-resistance by treatment with acriflavin. Nature, 189: 947.

Mitsuhashi, S., Harada, K., Hashimoto, H., Kameda, M. and Suzuki, M. (1962) Combination of two types of transmissible drug-resistance factors in host bacterium. J. Bact., 84: 9-16.

Molholt, B. and Fraser, D. (1965) Reversal of the restriction for host modified T2 and T4 DNA upon conversion of non-permissive Escherichia coli to spheroplasts. B.B. Res. Comm., 19: 571-575.

Molina, A. M. (1964) Genetic elements with cytoplasmic location controlling drug and phage T1 resistance in Enterobacteriaceae. Transfer of resistance factors by conjugation. Giornale di microbiologia, 12: 107-120.

Molina, A. M., Schito, G. C., Calegri, L. and Romanzi, C. A. (1965) Elementi genetici a localizzazione citoplasmatica controllanti la resistenza ad antibiotici e la restrizione del fago T1 nelle Enterobacteriaceae. IV. Trasferimento per coniugazione dei determinanti genetici in un ceppo streptomicino-dipendente di E. coli B. Boll. Ist. Sieroter, Milanese, 44: 326-328.

Monk, M. (1964) Colicinogeny in Escherichia coli K12. Ph.D. Thesis, University of London.

Newcombe, H. B. (1949) Origin of bacterial variants. Nature, 164: 150-151.

Nishimura, Y., Ishibashi, M., Meynell, E. and Hirota, Y. (1967) Specific piliation directed by a fertility factor and a resistance factor of Escherichia coli. J. gen. Microbiol., 49: 89-98.

Nisioka, T., Mitani, M. and Clowes, R. (1969) Composite circular forms of R factor deoxyribonucleic acid molecules. J. Bact., 97: 376-385.

Nisioka, T. and Ozeki, H. (1968) Early abortive lysis by phage BF23 in Escherichia coli K12 carrying the colIb factor. J. of Virology, 2: 1249-1254.

Okamoto, S. and Suzuki, Y. (1965) Chloramphenicol-, dihydrostreptomycin- and kanamycin-inactivating enzymes from multiple drug-resistant Escherichia coli carrying episome 'R'. Nature, 208: 1301-1303.

Ozeki, H., Howarth, S. and Clowes, R. (1961) Colicine factors as fertility factors in bacteria. Nature, 190: 986-989.

Paigen, K. and Weinfeld, H. (1963) Co-operative infection by host-modified lambda phage. Virology, 19: 565-572.

Pearce, L. E. and Meynell, E. (1968) Specific chromosomal affinity of a resistance factor. J. gen. Microbiol., 50: 159-172.

Pittard, J. (964) Effect of phage-controlled restriction on genetic linkage in bacterial crosses. J. Bact., 87: 1256-1257.

Ralston, D. J. and Kreuger, A. P. (1954) The isolation of a staphylococcal phage variant susceptible to an unusual host control. J. Gen. Physiol., 37: 685-716.

Revel, H. R., Hattman, S. and Luria, S. E. (1965) Mutants of bacteriophages T2 and T6 defective in α-glucosyl transferase. B.B. Res. Comm., 18: 545-550.

Romero, E. and Meynell, E. (1969) Covert *fi*⁻ R factors in *fi*⁺ R⁺ strains of bacteria. J. Bact., 97: 780-786.

Scaife, J. and Gross, J. D. (1962) Inhibition of multiplication of an F-lac factor in Hfr cells of Escherichia coli K12. B.B. Res. Comm., 7: 403-407.

Schell, J. and Glover, S. W. (1965) The nature of the restriction of host-modified phages by non-accepting hosts. Antonie van Leeuwenhoek, 31: 471.

Schell, J. and Glover, S. W. (1966a) The effect of heat on host-controlled modification of phage λ in Escherichia coli K(P1) J. gen. Microbiol., 45: 61-72.

Schell, J. and Glover, S. W. (1966b) On the localization of a factor responsible for host-controlled restriction in Escherichia coli K(P1). Genet. Res., 7: 277-279.

Shedlovsky, A. and Brenner, S. (1963) A chemical basis for the host-induced modification of T-even bacteriophages. Proc. natn. Acad. Sci., U.S.A., 50: 300-305.

Stacey, K. A. (1965) Intracellular modification of nucleic acids. Brit. Med. Bull., 21: 211-

Stacey, K. A., Symonds, N., Glover, S. W. and Schell, J. (1963) The chemical changes of phage DNA involved in host-induced modification. Struktur und funktion des genetischen materials. Ed. H. Stubbe.

Siccardi, A. G. (1966) Colicin resistance associated with resistance factors in Escherichia coli. Genet. Res., 8: 219-228.

Stocker, B. A. D., Smith, S. M. and Ozeki, H. (1963) High infectivity of Salmonella typhimurium newly infected by the colI factor. J. Gen. Microbiol., 30: 201-221.

Strobel, M. and Nomura, M. (1966) Restriction of the growth of bacteriophage BF23 by a Colicin I (ColI-P9) factor. Virology, 28: 763-764.

Sugino, Y. and Hirota, Y. (1962) Conjugal fertility associated with resistance factor R in Escherichia coli. J. Bact., 84: 902-910.

Sundararajan, T. A., Rapin, A. M. C. and Kalckar, H. M. (1962) Biochemical observations on E. coli mutants defective in uridine diphosphoglucose. Proc. natn. Acad. Sci., U.S.A., 48: 2187-2193.

Symonds, N., Stacey, K. A., Glover, S. W., Schell, J. and Silver, S. (1963) The chemical basis for host induced modification in phage T2. B.B. Res. Comm., 12: 220-222.

Takano, T., Watanabe, T. and Fukasawa, T. (1966) Specific inactivation of infectious λ DNA by sonicates of restrictive bacteria with R. factors. B.B. Res. Comm.,25: 192-198.

Tomoeda, M., Inuzuka, M., Kubo, N. and Nakamura, S. (1968) Effective elimination of drug resistance and sex factors in Escherichia coli by sodium dodecyl sulfate. J. Bact., 95: 1078-1089.

Uetake, H., Toyama, S. and Hagiwara, S. (1964) On the mechanism of host-induced modification. Multiplicity activation and thermolabile factor responsible for phage growth restriction. Virology, 22: 202-213.

Unowsky, J. and Rachmeler, M. (1966) Mechanism of antibiotic resistance determined by resistance transfer factors. J. Bact., 92: 358-365.

Watanabe, T. (1963) Episome-mediated transfer of drug resistance in Enterobacteriaceae. VI. High-frequency resistance transfer system in Escherichia coli. J. Bact., 85: 788-794.

Watanabe, T. and Fukasawa, T. (1961a) Episome-mediated transfer of drug resistance in Enterobacteriaceae. I. Transfer of resistance factors by conjugation. J. Bact., 81: 669-678.

Watanabe, T. and Fukasawa, T. (1961b) Episome-mediated transfer of drug resistance in Enterobacteriaceae. III. Transduction of resistance factors. J. Bact., 82: 202-209.

Watanabe, T. and Fukasawa, T. (1962) Episome-mediated transfer of drug resistance in Enterobacteriaceae. IV. Interactions between resistance transfer factor and F-factor in Escherichia coli K12. J. Bact., 83: 727-735.

Watanabe, T., Fukasawa, T. and Takano, T. (1962) Conversion of male bacteria of Escherichia coli K12 to resistance to f phages by infection with the episome "Resistance Transfer Factor". Virology, 17: 217-219.

Watanabe, T., Nishida, H., Ogata, C., Arai, T. and Sato, S. (1964a) Episome-mediated transfer of drug resistance in Enterobacteriaceae. VII. Two types of naturally occuring R factors. J. Bact., 88: 716-726.

Watanabe, T., Nishida, H., Arai, T., Sato, S. and Ogata, C. (1964b) Restriction and host-induced modification of phages λ and T1 by certain types of R factors in Escherichia coli. Struktur und funktion des genetischen materials. Ed. H. Stubbe.

Watanabe, T. and Okada, M. (1964) New type of sex factor-specific bacteriophage of Escherichia coli. J. Bact., 87: 727-736.

Watanabe, T., Takano, T., Arai, T., Nishida, H. and Saito, S. (1966) Episome mediated transfer of drug resistance in Enterobacteriaceae. X. Restriction and modification of phages by fi⁻ R factors. J. Bact., 92: 477-486.

Wollman, E. L. and Jacob, F. (1955) Sur le mécanisme du transfert de matériel génétique au cours de la recombinaison chez Escherichia coli K12. C.R. Acad. Sci. Paris, 240: 2449-2451.

Wollman, E. L. and Jacob, F. (1958) Sur les processus de conjugaison et de recombinaison chez Escherichia coli. V. Le méchanisme du transfert de matériel génétique. Ann. Inst. Pasteur, 95: 641-666.

Wood, W. B. (1965) Mutations in E. coli affecting the host-controlled modification of bacteriophage λ. Path. Microbiol., 28: 73-76.

Wood, W. B. (966) Host specificity of DNA produced by Escherichia coli: Bacterial mutations affecting the restriction and modification of DNA. J. Mol. Biol., 16: 118-133.

Yamada, T., Tipper, D. and Davies, J. (1968) Enzymic inactivation of streptomycin by R factor-resistant Escherichia coli. Nature, 219: 288-291.

Yoshikawa, M. and Akiba, T. (1962) Studies on transferable drug resistance in bacteria. IV. Suppression of plaque formation of phages by the resistance factor. Jap. J. Microbiol., 6: 121-132.

Zimmerman, S. B., Kornberg, S. R., and Kornberg, A. (1962) Glucosylation of deoxyribonucleic acid. II. α-glucosyl transferases from T2- and T6-infected Escherichia coli. J. Biolog. Chem., 237: 512-518.

Zinder, N. D. (1960) Hybrids of Escherichia and Salmonella. Science, 131: 813-815.

RESTRICTION AND MODIFICATION OF BACTERIOPHAGES BY R^+ STRAINS OF ESCHERICHIA COLI K12

D. Bannister and S. W. Glover

M.R.C. Microbial Genetics Research Unit,
Hammersmith Hospital, Ducane Road, LONDON, W.12.

Received February 23, 1968

It has been previously reported by Watanabe and Okada (1964), Watanabe *et al.* (1964, 1966) that the *fi*$^-$ class of resistance transfer factors (R factors) restrict the growth of certain bacteriophages. Siccardi (1966) showed that restriction of phages BF23 and W31 was associated with *col I* resistance conferred by R factors, and that this was not confined to the *fi*$^-$ R factors alone. We have surveyed a total of 151 R factors (71 *fi*$^+$, 65 *fi*$^-$ and 15 for which the *fi* character was not determined) in the *Escherichia coli* K12 strain J5-3 F$^-$ *pro met* for restriction of the phages: λ, ø80, P2, P1, BF23, W31, T3 and ø1. The J5-3R$^+$ strains were kindly provided by Dr. Naomi Datta. The initial survey was carried out by spotting serial hundred fold dilutions of the J5-3 grown phage onto lawns of the R$^+$ cultures. The efficiencies of plating (e.o.p.) were then measured accurately by plating out suitable dilutions of phage. Table 1 presents the results of this survey. Of the 151 R factors tested, 59 (33 *fi*$^-$, 20 *fi*$^+$ and 6 others) restricted one or more of the phages. These 59 R factors can be divided into ten groups on the basis of the e.o.p. values shown in Table 1. It is interesting to note that some of these groups contain both *fi*$^-$ and *fi*$^+$ R factors.

Groups VI and IX show an e.o.p. of less than 1×10^{-3} for phage BF23 and a reduction in e.o.p. for phage W31. Six of the R factors in these two groups had been previously tested for colicin production and colicin resistance (Meynell, personal communication). All six were colicinogenic

TABLE 1.

Efficiency of plating of bacteriophages on R^+ strains of E. coli J5-3

R factor group	T3	W31	ø1	BF23	λvir	ø80	P2	P1	*No. of R factors fi⁻	fi⁺	not tested
I	1.0	1.0	1.0	1.0	4×10^{-4}	1×10^{-4}	3×10^{-5}	1×10^{-1}	-	1	-
II	1.0	1.0	1.0	1.0	2×10^{-2}	7×10^{-3}	6×10^{-1}	7×10^{-4}	3	6	-
III	1.0	1.0	1.0	1.0	$< 10^{-2}$	3×10^{-2}	1.0	1.0	1	3	-
IV	1.0	1.0	1.0	1.0	$< 10^{-2}$	1.0	1.0	1.0	-	-	1
V	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-3}$	3×10^{-1}	$< 10^{-2}$	1.0	1.0	1.0	3	-	-
VI	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-2}$	1.0	1.0	1.0	1	-	-
VII	8×10^{-1}	7×10^{-3}	7×10^{-1}	5×10^{-1}	1.0	1.0	1.0	7×10^{-7}	-	2	-
VIII	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-3}$	1×10^{-1}	1.0	1.0	1.0	1.0	2	1	1
IX	7×10^{-1}	5×10^{-1}	8×10^{-1}	$< 10^{-3}$	1.0	1.0	1.0	1.0	6	3	2
X	1×10^{-1}	1×10^{-1}	1×10^{-1}	1.0	1.0	1.0	1.0	1.0	17	4	2
others	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	32	51	9

The e.o.p. values are based on averages of at least two experiments with each member of a group. The e.o.p. is indicated as less than ($<$) a certain value in those cases where, because of abnormal plaque morphology, plaque counts were subject to considerable error.

*The fi character of the R factors was determined by Dr. Naomi Datta and Dr. Elinor Meynell.

for col Ib and col I resistant. Restriction of these phages associated with col I resistance has also been found by Siccardi (1966).

A number of e.o.p. values for phages T3, W31, Ø1 and BF23 reported in Table 1, though less than 1.0, are exceptionally high (1×10^{-1} to 8×10^{-1}). More commonly the e.o.p. of host modified phage on restricting host strains is reduced by several log. units (Lederberg 1957). However, Arber (1966) has shown that phage fd.B plates on B (P1) with an e.o.p. of 3×10^{-1}, and is host modified by it. In the examples cited in Table 1 there is some reduction in plaque size and, with the so-called female specific phages (T3, W31 and Ø1), there is a variation in plaque morphology (e.g. crenelation of the halo, or no halo). This effect would not be expected if, after the first round of infection, the phage was host modified and subsequent rounds of infection were normal. It is more likely that the reduction in e.o.p. and plaque size are due to one or more of several factors, for example reduction in burst size, inefficient adsorption, and slightly impaired transmission. Watanabe et al. (1966) have already reported a reduced burst size for T1 in an R^+ host.

In many instances where the e.o.p. recorded in the Table 1 is less than 1×10^{-2} and the phage is not host modified it has been possible to isolate mutants, which are no longer restricted, from plaques with normal morphology.

We have demonstrated host modification of phages λ, Ø80 and P2 in group I, and of λ, Ø80 and P1 in group II. Host modification of these phages is group specific in that phage grown on group I is still restricted by group II (and vice versa) and also in that phage grown on any member of group II now plates efficiently on any other member of the group, (Table 2). In addition, the phages λ, Ø80 and P1 grown on either group I or II, still plate inefficiently on the other restricting groups (see Table 1). Group II may be identical with the R factors found by Watanabe et al.(1964, 1966) which host modify λ, and an R factor which restricts but does not modify λ reported by them may belong to one of our groups V or VI.

Table 2.

Host modification of phages λ, ø80, P1 and P2 in *E. coli* J5-3R$^+$ strains

Phage	R factor		
	none	group I (1 R factor)	group II (9 R factors)
λ.J5-3	1.0	$4x10^{-4}$	$2x10^{-2}$
λ. group 1	1.0	1.0	$1x10^{-2}$
λ. group II	1.0	$2x10^{-4}$	1.0
ø80.J5-3	1.0	$1x10^{-4}$	$7x10^{-3}$
ø80. group 1	1.0	1.0	$4x10^{-3}$
ø80. group II	1.0	$1x10^{-4}$	1.0
P2.J5-3	1.0	$3x10^{-5}$	-
P2. group I	1.0	1.0	-
P1. J5-3	1.0	-	$7x10^{-4}$
P1. group II	1.0	-	1.0

The figures in the table represent approximate e.o.p. values. The e.o.p. for phages grown on group II are expressed as an average for all members of the group.

Acknowledgements

The authors wish to thank Dr. Naomi Datta and Dr. Elinor Meynell for the generous gift of the R factors used in this work and for the unstinted advice and unpublished information concerning them.

One of us (D.B.) wishes to thank the Medical Research Council for a Scholarship for Training in Research Methods.

References

Arber, W. (1966) J. Mol. Biol. 20, 483.
Lederberg, S. (1957) Virology 3, 496.
Siccardi, A. G. (1966) Genet. Res. Camb. 8, 219.
Watanabe, T. and M. Okada (1964) J. Bacteriol. 87, 727.
Watanabe, T., H. Nishida, C. Ogata, T. Arai and S. Sato (1964) J. Bacteriol. 88, 716.
Watanabe, T., T. Takano, T. Arai, H. Nishida and S. Sato (1966) J. Bacteriol. 92, 477.